Reintroduction of *Amsinckia grandiflora* to Stewartville

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Abstract

Amsinckia grandiflora Kleeb. ex Gray is known from only two locations within Site 300 of Lawrence Livermore Laboratory, approximately 14 miles east of Livermore, California. In recent years the largest population has fluctuated in size between 23 and 355 individuals having once been comprised of "thousands" in the mid 1960's. The other population, less than two miles away, had fewer than 25 individuals when discovered in the spring of 1988. Consequently, Amsinckia can be considered one of the most endangered plants in California and perhaps the nation. The recovery plan, drafted by the U.S. Fish and Wildlife Service, called for the establishment of four new Amsinckia populations within its historic range in order to reduce the probability of extinction. The present study is part of an effort to create those new populations.

Using existing data on the distribution and ecology of the species, Pavlik and Heisler (1988) characterized and evaluated the habitat of Amsinckia populations at Site 300, and conducted a search for similar habitat within historic range. A total of 12 finalist sites were identified, among them the steep hillsides in the vicinity of Stewartville within Black Diamond Mines Regional Preserve. Those hillsides support mesic annual grassland on soils of the Altamont-Fontana complex and are therefore, suitable habitat for Amsinckia.

Using methods developed on this and other endangered plants the present study attempted to; 1) reintroduce *Amsinckia grandiflora* to its historical locality near Antioch, California (the Stewartville 1 site), taking into account the genetic structure of nutlet source populations and its contribution to the new, resident population, 2) demographically monitor the new population, emphasizing plant survivorship and seed (= nutlet) production, and 3) conduct experiments to determine the effects of fire, grass clipping and a grass-specific herbicide on survivorship and seed production of the new *Amsinckia* population. The results could then be used to establish additional satellite populations of *Amsinckia grandiflora* and, hopefully, new populations of other endangered plants.

The reintroduction could be termed a success in its first year. After sowing 3,260 nutlets in a total of 20 experimental plots, the number of germinules produced during the 1989-90 growing seaon (November to April) was large (1774) and many (1101) survived to reproduce. From these plants, an estimated 35,800 resident nutlets were produced, indicating that the population has the potential of growing by an order of magnitude in its second year.

Annual grass cover was found to have no effect on *in situ* germination but it had a significant negative effect on mortality rates, survivorship to reproduction, plant size and reproductive output (nutlet production). Therefore, annual grass cover must be controlled in order to promote population growth and stability of this highly endangered plant. Grass cover was effectively manipulated by using fire or grass-specific herbicide (in this case Fusilade®). Burning significantly reduced mortality rates early in the growing season and significantly increased survivorship to reproduction and maximum plant size. Nutlet output per plant was higher in burn plots but the enhancement was not statistically significant. The effect of burning on nutlet output was diminished because of annual grasses that re-established themselves after the burn and grew vigorously late in the season. Spraying with Fusilade® had no effect on mortality rates or survivorship to reproduction, but it significantly increased plant size and, therefore, nutlet output per plant and per plot. The herbicide treatment effectively eliminated competition from annual grasses and greatly increased the reproductive output of *Amsinckia grandiflora*. Hand clipping of the grasses, however, apparently intensified competition later in the growing season for unknown reasons. *Amsinckia* plants in clipped plots were smaller and produced fewer nutlets than control plants, although the differences were not statistically significant. The results of this experiment indicate that livestock grazing could have a detrimental effect on *Amsinckia* populations even if the effects of trampling and direct consumption were minimal.

Nutlets from the more genetically variable Site 300 source did not demonstrate better demographic performance than those from the Davis source. Germination, mortality rates, survivorship to reproduction and nutlet output per plant were the same for all plants regardless of origin. Therefore, the rather small differences in alleles per locus, % polymorphic loci, and heterozygosity per locus (for the enzyme systems which were characterized electrophoretically) had no apparent effect on the fitness of individuals or the genetic structure of the new population (although more data are being

generated on the latter). There were, however, some consistent differences between the two sources in response to burning and reproductive phenology that could, after many generations, produce some significant effects at the population level.

New populations of *Amsinckia grandiflora* can be created in mesic annual grassland if the habitat is treated to minimize competition with annual grasses. The study demonstrates that we are not yet able to make very accurate predictions of the demographic characteristics of reintroduced populations or of the effects of certain treatments on the habitat. It stresses the need for additional experimental studies of rare plants and their habitats in order to generate basic data that can be practically applied to specific conservation efforts.

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This project owes much to the support of Ann Howald at the Endangered Plant Program. Back-breaking efforts and helpful criticisms were supplied by Johanna Wolgast, a student at Mills College, who was the cornerstone of the lab and field work. I also cannot say enough good things about Roger Epperson and his staff at Black Diamond Mines Regional Preserve. Rangers Louis Guzman, Carol Alderdice and Kathleen Young deserve special mention. The project as a whole could not have been accomplished, however, without the hard work and generous contributions of Karen Heisler, Erin Espeland, Barbara Leitner, Marjorie Nelson, Kevin Shea, Frances Whitman, Amy Weins, and Drs. James Affolter, Stephen Edwards, Ronald Kelley, Robert Ornduff, Daniel Nickrent, Daniel Pantone, Dean Taylor, and Steven Weller.

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Introduction

Amsinckia grandiflora [1] Kleeb. ex Gray is known from only two locations within Site 300 of Lawrence Livermore Laboratory, approximately 14 miles east of Livermore, California. In recent years the largest population (the "droptower" population) has fluctuated in size between 23 and 355 individuals (Figure 1), having once been comprised of "thousands" in the mid 1960's (Taylor 1987, R. Ornduff, UC Berkeley, personal communication 1989). The other population (the "Draney Canyon" population), less than two miles away, had fewer than 25 individuals when discovered in the spring of 1988 (Pavlik 1989). During March of 1990, the populations were smaller than in previous years and individual plants (mostly less than 15 cm tall) had produced only one or two nutlets each. Consequently, Amsinckia can be considered one of the most endangered plants in California and perhaps the nation. The recovery plan, drafted by the U.S. Fish and Wildlife Service, calls for the establishment of four new Amsinckia populations within its historic range in order to reduce the probability of extinction. The present study is part of an effort to create those new populations.

Using existing data on the distribution and ecology of the species, Pavlik and Heisler (1988) characterized and evaluated the habitat of Amsinckia populations at Site 300, and conducted a search for similar habitat within historic range (Site 300 to Antioch). Land use patterns and logistic factors that could effect the success of a reintroduction effort were also considered. A total of 12 finalist sites were identified, among them the steep hillsides in the vicinity of Stewartville within Black Diamond Mines Regional Preserve. Those hillsides support mesic annual grassland on soils of the Altamont-Fontana complex and are therefore, suitable habitat for Amsinckia.

[1] *Amsinckia grandiflora* will often be referred to by its generic epithet.

Using methods developed on this and other endangered plants (Pavlik 1987, Pavlik and Barbour 1988, Pavlik et al. 1988), the present study attempted to; 1) reintroduce *Amsinckia grandiflora* to its historical locality near Antioch, California (the Stewartville 1 site of Pavlik and Hiesler, 1988), taking into account the genetic structure of nutlet source populations and its contribution to the new, resident population, 2) demographically monitor the new population, emphasizing plant survivorship and seed (= nutlet) production, and 3) conduct experiments to determine the effects of fire, grass clipping and a grass-specific herbicide on survivorship and seed production of the new *Amsinckia* population. The experiments were designed to test the hypotheses presented in Table 1. The results can then be used to establish additional satellite populations of *Amsinckia grandiflora* and, hopefully, new populations of other endangered plants.

Table 1. Statement of the basic hypotheses to be tested in the experiments designed around the reintroduction of *Amsinckia grandiflora* to Stewartville.

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a) Annual grass competition has no effect on the demographic performance of *Amsinckia grandiflora.*

{Demographic performance will be measured using *in situ* germination, mortality rates, survivorship to reproduction, plant size and reproductive output}

b) Demographic performance cannot be affected by manipulating annual grass cover using fire, hand-clipping or a grass-specific herbicide.

c) Nutlets from the Site 300 source will not demonstrate better demographic performance than those from the Davis source as the result of genetic differences.

Methods and Materials

Site Selection and Microsite Evaluation

The process of selecting pilot sites for new *Amsinckia* populations (Figure 2) was described in detail by Pavlik and Hiesler (1988). Many factors were taken into consideration, some ecological (macroclimate, soil, exposure, community associates, habitat size and degree of disturbance), and others logistic (land use history, road access, property ownership). The selection of Stewartville 1 (ST1) was based on its high potential as habitat (mesic grassland climate on or near soils of the Altamont-Fontana complex), its public status as part of the East Bay Regional Park (EBRP) system (it lies within Black Diamond Mines Regional Preserve (BDMRP)), and the fact that it lies within the historic range of *Amsinckia grandiflora*.

The exact location of the reintroduction plot (the microsite) was determined from field and laboratory studies conducted in March and April, 1989. On March 15, 1989, a field survey of five potential microsites near Stewartville was conducted with the assistance of Ann Howald (Plant Ecologist, CDFG Rare Plant Project), Stephen Edwards (Director, Regional Parks Botanical Garden) Roger Epperson, (Head Ranger, BDMRP) and Kevin Shea (East Bay Regional Parks). Each of the five microsites met the major criteria for delineating reintroduction sites: 1) large enough to allow 1 X 1 meter quadrats nested within 2 X 2 meter treatment zones, separated by row and column spaces (access paths), 2) relatively homogeneous with respect to microhabitat factors (soil depth, slope, associated species, etc.), 3) conformed to standards for experimental design, with replicate quadrats of a treatment exposed to existing variability within the plot, 4) reasonable balance achieved between accessibility and potential for human disturbance, and 5) surrounded by suitable habitat, so as not to contrain population growth in the future.

At each of the five microsites (Stewartville high, Stewartville low, Oil Canyon, Oil Canyon 2 and Lougher Ridge), a list of dominant species was made and 2 bulk (-10 cm depth) soil samples taken. The soil was sealed in plastic bowls with tight-fitting lids to retain moisture. General characteristics of the site (elevation, aspect, exposure) were also noted. Estimates of standing crop at the Lougher Ridge microsite were made by harvesting all of the above-ground plant material in four replicate 0.25 m^2 circular

quadrats. These were oven-dried to constant weight and compared with similar samples obtained from the droptower and Draney Canyon populations at Site 300, collected on 3/30/89. Standing crop gave an estimate of leaf canopy density and, therefore, competition between *Amsinckia* and the dominant species (annual grasses) at each microsite. This should be roughly equivalent between Site 300 and the reintroduction microsite.

Bulk soil samples were brought back to the lab and each was subsampled to obtain 4 samples (60 to 80 g) per site. These were used to determine oven-dry (4 days @ 80C), gravimetric soil moisture content. Percent soil moisture from these localities would eventually be compared to that of bulk samples obtained from the droptower and Draney Canyon populations at Site 300, collected on 3/30/89. Soil moisture content during the time of maximum flower and seed production (mid to late March) is probably an important index of habitat suitability for *Amsinckia*. Presumably, the Stewartville microsite with a soil moisture content similar to that found supporting active populations at Site 300 would be best for the reintroduction.

Soil from the five Stewartville microsites was also used to germinate nutlets and grow *Amsinckia* seedlings under greenhouse conditions. Three replicate 3" peat pots were filled with soil from the microsites and sown with three nutlets each (due to lack of expendable material of this taxon). Another three pots, filled with UC potting mix, were also sown and included for comparative purposes. The nutlets were a mixture of color morphs (white, gray and dark gray) obtained from the 1987 UC Davis crop or the 1988 UCB crop, but all were of large size (more than 2 mg) and had a typical, teardrop shape. Drawings of nutlet placement within the pots provided a record of their color morph and origin. The nutlets were covered by 1 cm of the same soil, watered and tamped down to insure good contact. The pots were moved to the Mills Greenhouse and given distilled water every day. Germination, survivorship and shoot dry weight (after 30 days of growth) were used as indicators of the suitability of the soil for *Amsinckia*. An index of *Amsinckia* performance was constructed from the sum of mean germination (% of total nutlets 10 days post-sowing), mean survivorship (% surviving 30 days post-emergence) and relative growth (% of mean shoot dry weight of the plants grown in potting mix (relative growth = 100%) 30 days post-emergence) for each soil type. The higher the index, the better the *in vitro* demographic performance and the greater suitability of the microsite soil for supporting a population of *Amsinckia*.

leaves, roots and stems were cut into pieces and combined with 80 to 100 µl of iced micromega buffer (see Nickrent 1989 for details). A polytron homgenizer (Brinckman Instruments) was used at high speed for up to 30 sec to insure a smooth, green homogenate. The homogenate was centrifuged at 10,000 G for 15 min at 4 C. The supernatant containing the soluble protein fraction from a single individual was decanted into a microcentrifuge tube and immediately stored at -90 C.

Electrophoresis. Two buffer systems were chosen for the starch gel electrophoresis (Table 2) in an attempt to resolve 20 different enzyme systems. Details of the buffers, starch gel, electrophoretic procedures and gel staining are found in Nickrent (1989).

Table 2. Enzyme systems and their respective gel buffers used for starch gel electrophoresis of *Amsinckia grandiflora* extracts. Nomenclature follows Conkle et al. (1982) in accordance with EC reference system.

Ridgeway pH 8.0	Histidine citrate pH 6.0
enzymes successfully resolved	
PGM = phosphoglucomutase	ACO = aconitase
PGI = phosphoglucoismerase	MDH = malate dehydrogenase
ALD = aldolase	IDH = isocitrate dehydrogenase
LAP = leucine amino peptidase	SKDH = shikimate dehydrogenase
GOT = glutamate oxaloacetate transaminase	6-PGD = 6-phosphogluconate dehydrogenase
G-3-PDH = glyceraldehyde 3-P dehydrogenase	
enzymes tried unsuccessfully	
MNR = menadione reductase	G-6-PD* = glucose-6-phosphate dehydrogenase
ADH = alcohol dehdrogenase	ACP = acid phosphotase
PER = peroxidase	AK = adenylate kinase
EST = beta esterase	
TPI* = triose phosphate isomerase	
CAT = catalase	

* very light banding

Determination of the Mixture of Founder Nutlets

Since genetic variability in small populations is often low (Waller et al. 1987) and can be ecologically restrictive (Hamrick et al. 1979, Schwartz 1986), it may be important to maximize allelic diversity in new populations to insure growth and persistence. In the case of *Amsinckia grandiflora*, allelic diversity will depend on the number of nutlets from each source population that germinate (and ultimately reproduce) in the field and the genetic constituency of those germinules. If the sources differ in germination potential (laboratory germination) and in their genetic diversity, then different mixtures of Site 300 and Davis nutlets will produce different population structures.

Laboratory germination and allozyme variability data were used to model the effects of different sowing mixtures (the ratio of the Site 300 to Davis nutlets) on population size and genetic heterogeneity. The principle effects would be on the number of germinules initially produced in each plot (due to differences in germination potential between the two sources) and on the number of carriers of low frequency alleles (recessives if one assumes Hardy-Weinberg equilibrium) among the germinules (due to differences in germination and the frequency of heterozygotes + homozygotes for infrequent alleles). Germinules that carry low frequency alleles as either hetero- or homozygotes are herein refered to as alternative allele carriers (AAC's). The practical constraint on maximizing the number of AAC's in a new population is the number of nutlets available from the most genetically heterogeneous source (in this case, Site 300). Nevertheless, it is beneficial to predict the expected number of AAC's so that additional compensations can be considered before the reintroduction begins (e.g. sowing additional seeds to improve the yield of germinules).

Although the model predictions will be presented in the results section, suffice it to say at this point that a 30/70 ratio of Site 300 to Davis germinules was chosen. This means that each sowing frame used to put nutlets in a treatment plot would have 30 wells devoted to Site 300 and 70 to Davis. A 30/70 ratio of germinules could only be obtained, however, with 100% germination of both sources. To compensate for differences in germination potential, a total of three Site 300 nutlets were sown into each of the 30 planting wells, compared to one of each Davis nutlet in each of the remaining 70 wells of a plot.

Amsinckia grandiflora (Pavlik 1988). The technique allows for estimates of nutlet output based on the sum of the inflorescence lengths of an *Amsinckia* plant ($r = 0.84$, $P<0.01$, $n = 30$) or shoot length ($r = 0.71$, $P < 0.01$, $n = 30$). For plants in the field, the latter was easiest to apply since shoot length (equivalent to maximum plant height above the soil) was readily measured for each plant in the plots at the time of maximum nutlet production (late March).

The relationship between shoot length and nutlet output per plant used in this study was developed by harvesting 18 individuals chosen to vary in size from among all of the Stewartville reintroduction plots (treatment and control and Site 300 and Davis plants were pooled). Plants were selected on April 9, 1990 after growth and nutlet production had essentially ceased. Maximum shoot length was measured (the entire range of 15.5 to 49.0 cm was included in the sample) and the plants were clipped at soil level, sealed in separate polyethylene bags and kept refrigerated until the remaining data were obtained two weeks later. Measurements of total inflorescence length and counts of the number of branches, inflorescences, flowers and nutlets were made in the lab. Inflorescences were removed from the vegetative portions of the plant by clipping immediately below the first flower. Each flower was examined for the presence of filled (good quality) nutlets which were then counted, removed, and placed in a pre-weighed envelope assigned to that individual plant. Nutlets from a single individual were weighed together and the average weight/nutlet was computed by dividing by the total number of nutlets. The number of ovules was estimated by multiplying flower number by 4 since each flower produces 4 single-ovuled nutlets (Ornduff 1976).

Linear and non-linear regressions were made using total shoot length and total inflorescence length (the sum of inflorescence lengths from a single plant) as the independent variable and nutlet output per plant as the dependent variable. The relationship from *in situ* plants with the highest regression coefficient was used to convert the height of each plant (= maximum shoot length) in every plot to to nutlet output at the peak of fruit set (March 19 and 20, 1990). Plot analyses were made by summing the nutlet output of all plants in a single treatment plot.

Evaluation of the treatments was made by comparing germination, mortality rates, survivorship to reproduction and nutlet output per plot between replicate experimental plots and the appropriate control plots. Statistical analysis of differences was made using analysis of variance (ANOVA) with arcsine transformation where appropriate.

Predictions Based on Existing Data

What is the expected yield of reproductive plants and nutlets produced *in situ* ? Table 3 attempts to predict maximum and minimum values based on laboratory studies of *Amsinckia* (Pavlik 1988) and field studies of other herbaceous taxa (Pavlik et al. 1988). It should be emphasized that the effects of the plot treatments and the values for survivorship and nutlet production amount to educated guesses. They were initially presented to convey the possible outcome of the reintroduction effort and the great uncertainty under which it was being conducted (note the broad ranges). They also allow an evaluation of the reintroduction and determine if additional populations could be established using the methods employed in the present study.

Table 3. Predictions of population size and nutlet yield for *Amsinckia grandiflora* reintroduced to Stewartville.

treatment	# of sown nutlets	% field germination min	% field germination max	expected range of live germinules	% field survivorship to repro	range of live adult plants	expected # nutlets per plant	total in situ nutlet yield
control[a]	800	20[b]	80[c]	160 to 640	20[b]	32 to 128	10[d]	320 to 1280
burn[e]	800	40	80	320 to 640	40	128 to 256	40[c]	5120 to 10240
fusilade[f]	800	40	80	320 to 640	30[g]	128 to 256	30[g]	3840 to 7680
clip[h]	800	40[b]	80	320 to 640	40[b]	128 to 256	40[c]	5120 to 10240
predicted total population		**germinules** = 1120 to 2560			**adult plants** = 416 to 896		**nutlets** = 14410 to 29440	

[a] = maximum competition from annual grasses, effects of organic soil layer
[b] = based on field studies by Pavlik et al. (1988) on unrelated herbaceous taxa
[c] = based on lab studies by Pavlik (1988) on Amsinckia grandiflora
[d] = based on lab studies by Pavlik (1988) and field studies by Taylor (1987)
[e] = low competition, higher soil nutrient levels, larger Amsinckia plants (Pavlik 1988)
[f] = low competition, ambient soil nutrient levels
[g] = anticipated effects of herbicide residuals
[h] = low competition, ambient soil nutrient levels, disturbed soil surface

Results and Discussion

Microsite Evaluation

A comparison of the five Stewartville microsites with the two Site 300 microsites showed that only the Lougher Ridge area was similar to existing *Amsinckia grandiflora* habitat in terms of soil moisture and presence of important floristic indicators (Table 4). Lougher Ridge had the highest March soil moisture and the only consistant presence of other *Amsinckia* species (mostly *A. intermedia*), *Lupinus albifrons* (common at Site 300) and native grasses. Standing crop was also determined to be intermediate between the Droptower and Draney Canyon subpopulations, indicating a similar competitive regime into which the species would be reintroduced.

Furthermore, nutlets placed in the five microsite soils and grown under greenhouse conditions had their overall best performance in the Lougher Ridge soil (Table 5). The Lougher Ridge soil allowed moderate germination, high survivorship and high relative growth and was surpassed only by potting mix in producing robust plants. The cause of poor performance in the other soils is not known with certainty, although their high clay content was observed to cause poor drainage and uneven moisture distribution within the pot. Lougher Ridge soil is somewhat sandier, especially below the 0.5 m depth *in situ* where it is almost pure sand.

These tests demonstrated the potential importance of careful microsite evaluation for rare plant reintroduction and established that Lougher Ridge was the best choice for our efforts.

Table 4. Comparison of potential reintroduction microsites in the Stewartville area with extant *Amsinckia grandiflora* sites at Site 300. Standing crop and March (1989) soil moisture are means ± SD (n = 4).

	standing crop (gm/0.25 m^2)	March soil moisture (%)	site supports populations of		
			Amsinckia species	*Lupinus albifrons*	native *Poa, Stipa*
Site 300					
Droptower	54.0 ± 17.0	24.7 ± 3.3	yes	yes	yes
Draney Canyon	16.3 ± 3.6	24.0 ± 6.4	yes	yes	yes
Lougher Ridge	26.3 ± 4.0	23.8 ± 0.6	yes	yes	yes
Stewartville low		10.2 ± 0.2	no	no	no
Oil Canyon 2		17.2 ± 0.5	yes	no	yes
Oil Canyon 1		21.9 ± 3.0	yes	no	yes
Stewartville high		14.3 ± 0.3	no	no	no

Table 5. Effects of microsite soil type on the *ex situ* germination, survivorship and growth of *Amsinckia grandiflora*. Means are based on 3 replicate pots of 3 nutlets each (Davis 87 source)

soil	10 day germination (%)	1 month survivorship (%)	relative growth (%)	performance index (%)
potting mix	88.9	88.7	100.0	277.6
Lougher Ridge	77.7	100.0	31.6	209.3
Stewartville low	66.6	100.0	8.2	174.8
Oil Canyon 2	88.9	66.6	6.9	162.4
Oil Canyon	44.4	100.0	13.5	157.9
Stewartville high	77.7	50.0	5.8	133.5

Characteristics of the Nutlets

Laboratory germination was relatively high, with 58.8% of the Davis and 30.8% of the Site 300 nutlets producing germinules after 10 days in constant darkness (Table 6). The germination of Site 300 nutlets was achieved despite the nearly 25 years that had passed since harvest from the field. However, the expected field germination would be less because of patchiness in the soil environment, poor seed-soil contact, and predation or disease (Pavlik et al. 1988).

Table 6. Laboratory germination of *Amsinckia grandiflora* nutlets (at 25 C) from two source populations, July 1989. Mean ± SD from 6 lots of 10 nutlets each.

	Site 300	Davis
germination (%)	30.8 ± 11.3	58.8 ± 19.9

As a whole, the 28 seedlings analyzed by electrophoresis had very low levels of allozyme variability at the 18 loci examined (Table 7). Compared to other dicot, annual, endemic and outcrossing taxa, *Amsinckia grandiflora* has fewer alleles per locus, a much lower percentage of polymorphic alleles and very low heterozygosity overall. It is likely that this species has passed through a recent selection bottleneck that effectively removed much of the variability in the gene pool of the population. A similar hypothesis has been advanced for the endangered *Pedicularis furbishiae* (Waller et al. 1987) and for *Howellia aquatilis* by Lesica et al. (1988), both of which show little or no allozyme variation. It is unclear how the lack of variation in such taxa will ultimately effect their conservation. We might expect that if genetic variability is important, then habitat restoration would have only a short-term, positive effect on population size.

Analysis of allozyme variability within each source population showed that the Site 300 nutlets had the highest level of polymorphism, followed by Mills and Davis (Table 8). Site 300 plants were polymorphic at the PGM, PGI, IDH and SKDH loci, while Mills and Davis showed variation only at PGM and LAP. Even without considering the relatively depauperate Mills and Davis populations, *Amsinckia* from Site 300 was still not as genetically variable as other endemic taxa (Table 7). For the purposes of this analysis, the genetic data on Davis and Mills sources will be combined and referred to only as Davis because of small sample sizes, similar history (both derived from Site 300 and subsequently cultivated) and apparent allelic similarity. The frequency of alternative allele carriers (ACC's) in the Site 300 nutlets was 41.7% compared to 28.6% for Davis nutlets. Because the seedlings were of the same age and grown under uniform conditions, it is reasonable to conclude that Site 300 and Davis source gene pools possessed different alleles. It might, therefore, be important to monitor germinules from different sources during the reintroduction in order to examine the possible effects of different genes on establishment and fecundity.

Table 7. Comparison of genetic variability at 18 loci in *Amsinckia grandiflora* seedlings ($n = 26$, all source populations combined) to plants with similar life history and taxonomic traits.

	mean number of alleles per locus	% polymorphic loci	mean heterozygosity per locus
Amsinckia grandiflora	1.13	13.0	0.034
Dicotyledonae	1.46	31.3	0.113
endemic taxa	1.43	23.5	0.086
annual taxa	1.72	39.5	0.132
outcrossing taxa	1.85	51.1	0.185

Data on dicots, endemics, annuals and outcrossers from Hamrick et al. (1979).

Table 8. Genetic variability at 18 loci in *Amsinckia grandiflora* seedlings derived from 3 nutlet source populations.

	mean number of alleles per locus	% polymorphic loci	mean heterozygosity per locus	polymorphic loci
Site 300 (n =12)	1.22	22.2	0.044	PGM-1, PGI-2, IDH-1, SKDH
Mills 1988 (n = 6)	1.11	11.1	0.040	PGM -1, LAP-1
Davis (n = 8)	1.06	5.6	0.017	PGM -1
All sources (n = 26)	1.13	13.0	0.034	

Determination of the Mixture of Founder Nutlets

Differences in laboratory germination and genetic variability among the principle source populations are thus apparent in the data given above. The model of germinule and ACC yield suggests that double-sowing of Site 300 nutlets would compensate for lower Site 300 germination (Figure 7) and that the expected frequency of ACC's would be about 30% (Figure 8) if 1600 Site 300 nutlets were used (in a 40/60 Site 300 to Davis ratio). Given the observation that field germination is often markedly less than laboratory germination (see Pavlik et al. 1988), it seemed likely that even the 30% figure would not be reached. As a result, the decision was made to use more Site 300 nutlets (1800 total) by triple-planting in a 30/70 configuration (meaning that 30% of the 2000 sowing wells would receive 3 site 300 nutlets and that 70% would receive 1 Davis nutlet). Simply increasing the proportion of Site 300 nutlets in the total founder population (e.g. to 20/80) was not considered desirable because of uncertainties surround the number of plants that would actually be produced *in situ* . If Site 300 germination was very low and few plants were established, the critical population size

for attracting pollinators and outcrossing might not be reached and the size of the second generation at Lougher ridge would be significantly reduced . For this reason it was decided that a large proportion of Davis nutlets should be sown even if that meant that fewer ACC's would be in the founder population and that intraspecific competition among triple-sown Site 300 plants would occur.

As revealed by subsequent demographic data, germination of both the Site 300 and the Davis nutlets was higher than expected (see below). This meant that double-planting would probably have been sufficient to attain a 30% yield of ACC's and that intraspecific competition between Site 300 plants in the same sowing wells could have been avoided. Fewer Site 300 nutlets would have been expended and possibly a larger second generation would have been produced (although interspecific competitive effects were probably much greater than the intraspecific effects). Future reintroductions that use Site 300 nutlets will, therefore, avoid multiple sowing. Whether or not the predicted yield of ACC's was realized in the Lougher Ridge population will be tested when electrophoretic data become available in Fall 1990.

Effects of the Plot Treatments on the Lougher Ridge Grassland

On December 4, approximately one month after the first good rains, live grasses constituted 44% of the total cover in the control plots with less than 10% bare ground showing (Table 9). The upper tips of the blades were more than 20 cm high and, with last year's dead thatch, cast deep shade on the emerging *Amsinckia* seedlings. In contrast, live cover by grasses was half as much in all of the treatment plots. Burn plots were much more open because the fire had removed much of the thatch, leaving only ash (also recorded as "dead" cover). Clip and fusil plots had less open ground and more grass thatch, either because clipping left the rootcrowns in place or because the herbicide killed grass shoots that were left standing, respectively. The low height of grasses and thatch in the burn and clip plots allowed light to reach the seedlings through much of the day. The standing live and dead grasses in the fusil plots, however, produced a shady light environment similar to the controls at this time. *Amsinckia grandiflora* and other forbs constituted a very low percentage of live cover in the December plots.

During the peak of reproduction, however, the grass cover in the control and burn plots was similar because grass propagules that survived the fire grew vigorously throughout the late winter and spring (Table 10). Grasses in the burn plots were as tall or taller than the flowering *Amsinckia* , although the total cover by *Amsinckia* was still twice that of the controls. Fusilade-treated plots were strikingly different from burn and control plots because their live cover was completely dominated by large, profusely-flowering individuals of *Amsinckia grandiflora* (Table 11), with a surprisingly lush "understory" of mostly native forbs (e.g. *Amsinckia lycopsoides, Claytonia perfoliata, Galium aparine, Lithophragma affine,* and *Triteleia laxa*). Live cover of the introduced annual grasses (and in one case, a small amount of native perennial grass cover) was effectively controlled by the herbicide, and this favored the native forbs including the target species *Amsinckia grandiflora* .

Were the differences in cover (and *Amsinckia* performance, see below) a direct result of physical differences in the plots caused by the treatments or were they an indirect result of differences in competitive regimes? Removing the grass canopy could affect plant performance by changing the climate around the seedlings. There was no significant effect of the treatments on temperatures within the plots, however, as monitored with soil and air sensors (Figure 9). Mean daily temperatures in the vicinity of *Amsinckia grandiflora* plants in different plots were the same throughout the growing season when comparisons were made between all treatment plots and controls (i.e. the slope of the correlation was near 1.0 with very little consistant deviation). Canopy removal could also affect the interception and storage of precipitation, especially after treatment. Soil moisture levels in the plots were not, however, significantly different early in the growing season (Table 12), indicating that the treatments did not alter the physical interception or storage of precipitation. These data argue against direct, purely physical effects of canopy removal by burning, clipping or herbicide treatment.

More likely, the influence of the grass canopy was biological, with competitive effects that cannot be fully elucidated by the data on hand. The canopy in the control plots certainly absorbed light that would otherwise by used by the seedlings of *Amsinckia* and other forbs. This effect would be most critical early in the growing season. Later, as surviving forbs emerged from the grasses and produced leafy canopies, the competition might shift below ground, making water and nutrients the limiting resources rather than light. Indeed, by January there was significantly more

moisture in the soils of the fusilade-treated plots, perhaps because they lacked a water-consuming grass canopy. Clip plots still had significant live grass cover (low but dense) and grasses reestablished in the burn plots were growing rapidly at this time. Therefore, the treatment effects on *Amsinckia grandiflora* were due to alterations of biological rather than physical factors in the plots, thus producing differences in competitive regimes.

Table 9. Effects of treatments on the vegetation within plots at Lougher Ridge, December 4, 1989. Cover was estimated in replicate 0.25 m^2 circular quadrats within all plots (n= 4 per treatment). AG = *Amsinckia grandiflora.*

	relative live cover (%)	foliage height (cm)	live cover by	relative dead cover (%)	litter height (cm)	litter composed of	absolute bare ground (%)
control	44.1 ± 6.9	15-25	annual grasses	58.9 ± 6.9	10-15	grass thatch	8.0 ± 4.0
burn	16.5 ± 7.3	0-5	AG	83.5 ± 7.3	0-5	ash	53.0 ± 10.8
clip	22.6 ± 6.5	5-10	annual grasses, AG	77.4 ± 6.5	5-10	grass thatch	24.0 ± 8.6
fusil	19.0 ± 6.4	15-25	annual grasses, AG	81.2 ± 6.4	10-15	grass thatch	6.0 ± 3.7

Table 10. Effects of treatments on the vegetation within plots at Lougher Ridge, March 30, 1990. Cover was estimated in replicate 0.25 m^2 circular quadrats within all plots (n = 4 per treatment). AG = *Amsinckia grandiflora.*

	relative live cover (%)	foliage height (cm)	live cover by	relative dead cover (%)	litter height (cm)	litter composed of	absolute bare ground (%)
control	84.1 ± 5.8	30-50	annual grasses	15.9 ± 5.8	10-25	grass thatch	7.0 ± 5.1
burn	82.0 ± 7.0	30-50	annual grasses, AG	18.0 ± 7.0	10-20	grass thatch	12.0 ± 9.3
clip	79.7 ±6.1	5-15	annual grasses	20.3 ± 6.1	5-15	grass thatch	6.0 ± 2.0
fusil	80.7± 10.0	30-50	AG, other forbs	19.3 ± 10.0	10-20	grass thatch	10.0 ± 6.0

Table 11. Composition of the live cover within the Lougher Ridge plots, March 30, 1990. Cover was estimated in replicate 0.25 m^2 circular quadrats.

		live cover by			
	relative live cover (%)	grasses (%)	*Amsinckia grandiflora* (%)	forbs (%)	cover dominants
control	84.1 ± 5.8	69.2 ± 6.0	8.6 ± 2.8	6.4 ± 2.1	*Avena, Bromus, Hordeum*
burn	82.0 ± 7.0	54.2 ± 3.8	22.0 ± 4.7	5.8 ± 3.5	*Avena, AG, Marah*
clip	79.7 ±6.1	60.6 ± 10.3	10.2 ± 4.2	9.0 ± 4.4	*Avena, Hordeum, Erodium*
fusil	80.7± 10.0	1.1 ± 2.2	60.8 ± 12.9	18.8 ± 8.0	*AG, Claytonia, Galium*

Table 12. Effects of treatments on gravimetric soil moisture within the Lougher Ridge plots. Means ± SD within a column followed by the same letter are not significantly different (ANOVA, $P<0.05$).

	gravimetric soil moisture (%) on		
	Nov 16	Nov 30	Jan 4
control	17.3 ± 1.3[a]	32.1 ± 2.8[a]	24.4 ± 3.3[a]
burn	16.4 ± 1.9[a]	29.2 ± 2.8[a]	22.9 ± 2.5[a]
clip	17.9 ± 1.5[a]	30.3 ± 1.4[a]	24.3 ± 3.5[a]
fusil	19.0 ± 1.9[a]	28.3 ± 2.0[a]	29.7 ± 2.8[b]

Demographic Monitoring of the New Population

Germination

The first significant rains fell during October 22-24, with 38.1 mm received by the Lougher Ridge plot (Figure 10). The nutlets, sown only 4 days before, began to germinate immediately. On October 29 the first complete census was taken and it found that more than 30% of the sown nutlets had already germinated. Eight days later (November 6) more than 50% of the sown nutlets had germinated, constituting 90% of all germination that was to occur during the winter of 1989-90. There were no significant differences in the rate of germination among the treatment and control plots, although there was a slight delay in the burn plots during the first 9 days. Nutlets continued to germinate sporadically thoughout the growing season, with the last germinules recorded on February 18, 1990, 120 days after sowing.

Total *in situ* germination (% of nutlets sown) during the October 29 to February 18 period was higher than expected (based on lab germination), with 43% of the Site 300 and 70% of the Davis nutlets finally emerging (Table 13). The differences between the two sources were consistent among plots, reflecting age-specific rather than environmental effects on germination. Even the passage of fire across the nutlets in the burn plots had no significant effect, although slightly higher Site 300 and slightly lower Davis germination were observed. As a result, total germination of both nutlet subpopulations averaged between 54 and 55% regardless of treatment and germinule density was equivalent in all of the study plots at the beginning of the experiment.

Population Growth and Mortality

The entire Lougher Ridge population grew rapidly, attaining a maximum of 1774 live plants in germinule, seedling and juvenile stages. Totals of 443, 443, 456 and 432 individuals were found within the control, burn, clip and fusil plots, respectively, during the entire growing season. Because of even germination, each plot had 70 to 80 plants initially, with an average of ~40% being from the Site 300 source (Figure 11). There were no statistically significant effects of competition treatments on the proportion of live

Table 13. Total *in situ* germination (%, 29 October 1989 to 18 February 1990) of *Amsinckia grandiflora* nutlets as a function of plot treatment and source. Values (mean ± SD) in a column were not statistically different at $P < 0.05$ (ANOVA, arcsine transformed data).

	Site 300	Davis	both sources
control	41.8 ± 10.0	72.9 ± 9.9	55.4 ± 5.2
burn	49.5 ± 11.9	62.9 ± 12.2	55.4 ± 9.9
clip	40.9 ± 6.4	70.1 ± 4.6	54.1 ± 4.8
fusil	38.7 ± 10.3	73.7 ± 8.0	54.0 ± 8.1

plants that were derived from different nutlet sources, although there was a consistant, higher proportion of Site 300 plants in the burn plots (~ 50%) from mid-November until peak flowering in mid-March. The burn treatment may have provided better conditions for the Site 300 genotypes or perhaps the nutlets sown into the burn plots simply had a higher propensity to germinate (the outcome reflected a bias in the distribution of nutlets among plots). Although care was taken to allocate nutlets randomly, it should be noted that differences in the number of Site 300 plants among plots were observed from day 9 on. Therefore, it seems unlikely that enough environment-gene interaction could have taken place to select against Site 300 plants in the control, clip and fusil plots. In other words, the enrichment of the burn plots in Site 300 plants was the result of a random but generally higher rate of germination of Site 300 nutlets.

Although the first seedling deaths were detected within 17 days after sowing, they were not common until 27 days (mid-November) and later (Figure 12). At that time there were significant differences in mortality among plots. The average mortality rate in the control plots (~ 9 % per week) was more than twice that in the burn and clip plots, but statistically equivalent (by ANOVA) to that in the fusil plots. A significant difference between control and burn plots was also found in early December but not afterwards. Thus, the treatments (burn, clip) which minimized *Amsinckia* - grass interactions during

this time effectively reduced mortality of seedlings and young, established plants. The Fusilade treatment, since it was not administered until after the grasses had emerged in mid-November, had no effect on seedling survival. Afterwards, mortality rates in all plots declined to 1- 3% and then tended to rise slowly towards the late spring. There was no differential mortality between plants derived from Site 300 and Davis sources, indicating that neither genetic differences nor triple sowing (producing intraspecific competition) were important during the early, non-reproductive stages of the population.

The causes of mortality were not always clear. In some cases, germinules and seedlings vanished between census dates and their deaths could not be assigned to a particular stress category. However, it was often the case that we could directly observe evidence of water stress (wilting of leaves), grazing by microherbivores (chewed leaves, cotyledons and stems), and light or nutrient deficiency (chlorosis). Despite the fact that this was another drought year, only a small percentage of live plants were wilted during the entire growing season, ranging between 2 and 6% among all plots (Table 14). Grazing was much more prevalent, with at least 30% of all live plants losing tissue. Grazing effects were much easier to ascertain when the plants were small, so perhaps these estimates are understated for the growing season as a whole. There were no treatment or source effects on stress due to wilting and grazing . Chlorosis, however, was much more common in the control and fusil plots and was lowest in the burn plot, especially when the plants were young and small (the first 42 days). This suggests that the grass canopy present in both the control and fusilade plots had its effect on seedling mortality through competition for light and/or mineral nutrients at this time.

Flowering and Nutlet Output

Inflorescences of *Amsinckia grandiflora* were first observed on January 4, 1990, 76 days after sowing. A total of 3 control, 7 burn, 2 clip and 4 fusilade plants (1.1 % of all live plants) had tightly coiled, unopened flower buds with characteristic long, dark brown hairs (*A. intermedia* buds had shorter, tawny or gray hairs.) Most were found in the burn plots (7 of 16, = 44%) and most were from Site 300 nutlets (13 of 16, = 81%). By January 25 (day 97), 9 control, 24 burn, 18 clip and 32 fusil plants (5.6% of all live plants) had inflorescences, and 4 of these had open flowers.

Table 14. Stress factors in the treatment plots. Each value is the percent of the total live individuals during the growing season (November to April) that exhibited wilting, tissue loss due to micrograzers or were chlorotic (yellow) due to etiolation or nutrient deficiency.

	% of plants which were		
	wilted	grazed	chlorotic
control	6.5	35.4	31.6
burn	1.8	31.8	9.3
clip	3.3	44.5	16.0
fusil	6.2	29.6	23.4

All 4 of the plants with open flowers were Site 300 plants and all 4 were thrums. The earliest flower formation was seen, therefore, in Site 300 plants (especially if they were thrums) in the burn and Fuscilade-treated plots. This pattern was accentuated by February 18 (day 120), when 70% of all live plants had inflorescences. Of all live plants with open flowers at that time (a total of 19), 15 (= 80%) were from Site 300 nutlets, 16 (84%) were thrums and 17 (90%) were found in the burn plots. Although no plants with open flowers were in the fusil plots, 78% had inflorescenses compared with 82% in the burn plots, 60% in the control plots and 56% in the clip plots.

The peak of flowering was reached in mid-March (day 150), when 1101 out of 1310 living plants (84%) had open flowers and/or inflorescences with flowers undergoing anthesis (orange petals expanding beyond the calyx lobes). These were regarded as the reproductive plants, those likely to set nutlets before the end of the growing season. The remaining 198 plants were unlikely to reproduce because they were small (generally less than 16 cm tall and unbranched) and either had no inflorescences (25 of the 1310, or 2 %) or had new, tightly coiled inflorescences with no sign of impending anthesis (173 or 13 %). Reproductive plants in the burn plots (339) outnumbered those in the control, clip and fusil plots (191, 289 and 282, respectively). In mid-March,

however, plants from Site 300 nutlets were no more likely to be reproductive than plants from Davis nutlets.

The burn plots contained significantly more reproductive plants and, therefore, had higher survivorship to reproduction (expressed as a percentage of germinated nutlets) when compared to controls (Table 15). The 75% reproductive survivorship compares very well with the 83% reported by Pavlik (1988) for pampered, greenhouse-grown plants. Clipping and Fusilade did not significantly improve either of these, indicating that only the low seedling mortality rates observed in the burn plots (Figure 12) were of some demographic consequence to this annual plant. There was no treatment effect on pin/thrum ratio, which averaged 1.36 in all plots. Over several years at Site 300, Ornduff (1976) reported a range of 1.0 to 2.0, while Taylor (1987) found 0.75 to 1.2. The ratio of the reintroduced population at Lougher Ridge, therefore, is similar to that of the wild population and has the potential for reaching a stable equilibrium over several generations.

Table 15. Treatment effects on population size, survivorship to reproduction and pin/thrum ratio of *Amsinckia* (all sources) during the period of maximum flowering (mid-March 1990). Values (mean ± SD, n = 5) in a column followed by the same letter are not statistically different (P<0.05, ANOVA, arcsine transformed % and ratios).

	mean # of repro plants per plot	survivorship to reproduction (% of germ)	pin / thrum ratio
control	38.6 ± 15.8^{a}	42.7 ± 16.5^{a}	1.38 ± 0.33^{a}
burn	67.2 ± 19.8^{b}	75.3 ± 11.6^{b*}	1.40 ± 0.36^{a}
clip	57.8 ± 16.5^{a}	63.1 ± 12.0^{a}	1.27 ± 0.82^{a}
fusil	56.4 ± 15.6^{a}	64.4 ± 10.8^{a}	1.40 ± 0.49^{a}

* Different at P < 0.01

There was no differential reproductive survivorship of plants derived from Site 300 nutlets (Table 16). Control, clip and fusil plots had slightly fewer Site 300 plants while burn plots had slightly more, but the differences were not significant. There was no significant pattern in pin/thrum ratio of Davis and Site 300 plants.

Table 16. Treatment effects on population size, survivorship to reproduction and pin/thrum ratio of *Amsinckia* (for Davis and Site 300 sources) during the period of maximum flowering (mid-March 1990). Values (means, n = 5) in a column followed by the same letter are not statistically different ($P<0.05$, ANOVA, arcsine transformed % and ratios).

	mean # of repro plants per plot		survivorship to reproduction (% of germ)		pin / thrum ratio	
	Davis	Site 300	Davis	Site 300	Davis	Site 300
control	22.6[a]	15.6[a]	45.4[a]	38.9[a]	1.17[a]	2.11[a]
burn	33.6[a]	35.0[a]	75.1[b]	76.1[b]	1.44[a]	1.40[a]
clip	37.8[a]	20.0[a]	68.3[a]	55.5[a]	1.26[a]	1.47[a]
fusil	35.6[a]	20.8[a]	70.5[a]	59.5[a]	1.22[a]	1.40[a]

The output ~~of~~ nutlets by individual plants at Lougher Ridge was linearly related (Table 17) to the sum of the inflorescence lengths (Figure 13) and shoot length (Figure 14). The largest plants (with shoot lengths ranging from 35 to 50 cm) produced between 150 and 182 nutlets each. Ovule production was also related to the sum of the inflorescence lengths (Figure 13), but larger plants were not more efficient than smaller ones in converting ovules into nutlets (i.e. the slope of the sum of inflorescence lengths vs. reproductive efficiency ~0 and P = n.s., Table 17). Typically, medium to large plants had reproducive efficiencies (nutlet/ovule ratios) around 0.20. Maximum reproductive efficiency was 0.34, which compares well to the 0.30 reported for plants at Site 300 (Ornduff 1976) and exceeds the 0.22 reported for greenhouse-grown plants (Pavlik 1988).

Table 17. Linear correlations between various measures of plant size and nutlet output, ovule output or reproductive efficiency per individual *Amsinckia grandiflora* from the Lougher Ridge population, March 19, 1990. **Bold type** indicates the relationship shown in Figures. Data on 1988 garden-grown plants provided for comparative purposes. ns = not significant, Σ inflor lgth = sum of the lengths of all inflorescences, repro eff = reproductive efficiency

plants	n	X	Y	slope	intercept	r	P
Lougher Ridge 1990							
all plants	**18**	**Σ inflor lgth (cm)**	**# nutlets**	**2.507**	**-5.926**	**0.95**	**<0.01**
Davis	12	" "	" "	2.426	-6.186	0.95	<0.01
Site 300	6	" "	" "	3.288	-14.101	0.98	<0.01
all plants	**18**	**shoot length (cm)**	**# nutlets**	**4.600**	**-79.248**	**0.77**	**<0.01**
all plants	**18**	**Σ inflor lgth (cm)**	**# ovules**	**10.949**	**7.232**	**0.99**	**<0.01**
all plants	18	Σ inflor lgth (cm)	repro eff	0.001	0.144	0.43	ns
Garden 1988 (Pavlik 1988)							
all plants	29	Σ inflor lgth (cm)	# nutlets	1.129	-0.301	0.84	<0.01
all plants	30	shoot length (cm)	# nutlets	2.014	-45.971	0.71	<0.01
all plants	30	Σ inflor lgth (cm)	# ovules	7.708	102.332	0.93	<0.01
all plants	30	Σ inflor lgth (cm)	repro eff	0.001	0.055	0.44	<0.05

A total of 35,768 nutlets were produced by the 1101 reproductive individuals of *Amsinckia grandiflora* at Lougher Ridge, Stewartville by the end of March, 1990. This estimate was obtained by calculating the nutlet output of each and every plant in all plots using its measured shoot length (March 19 and 20) and the equation shown in Table 17. Because a total of 3,460 founder nutlets were input to the site, the seed bank population of *Amsinckia grandiflora* was amplified by about a factor of 10. Approximately 40% of the resident nutlets were derived from the Site 300 source (the proportion of Site 300 plants in the reproductive population), as differential survivorship (Table 16) and differential nutlet output (Table 20 below) were not detected. For the most part, the resident nutlets were allowed to disperse on their own at Lougher Ridge, except in the five most productive treatment plots (all Fusilade-treated). After mid-April, nutlets remaining on plants in those plots were collected, bagged according to plot and brought back to the lab at Mills. These have been stored open (to allow drying) at room temperature (to avoid cold temperature damage during the post-ripening period) and will be returned to the vicinity of their respective plots when new, unoccupied portions of adjacent habitat are treated in October 1990. The purpose of this intervention was to avoid exceedingly high densities of nutlets, and ultimately, germinules, that would accentuate intraspecific competition during the early phases of the 1990-91 population. This was not done for other treatment plots where nutlet outputs were not as high.

The burn and herbicide treatments significantly enhanced one or more measures of plant size and, consequently, the estimates of nutlet production (Table 18). Mean maximum shoot length was greater in both the burned and fusilade plots, while mean shoot length was greater only in the fusil plots. Burning alone did not release all individuals from competition because there were many small, presumably suppressed individuals in the burn plots along with the larger dominant ones. Intraspecific competition within the more dense burn plots (Table 15) cannot be ruled out, but interspecific competition was also significant because; 1) cover by grasses in the burn plots approached that in the controls during March (Table 11), and 2) fusil plots had *Amsinckia* densities similar to that in the burn plots but with significantly greater mean plant size in the absence of grasses. In other words, grass caryopses that survived the autumn fire were able to take advantage of the plot conditions, achieve large sizes, and be effective competitors by spring. Therefore, *Amsinckia* plants in the burn plots had only a slight enhancement (statistically insignificant, $P<0.10$) of nutlet output per plant

compared with the controls, but those in fusil plots produced more than three times the number of nutlets as controls. The mean nutlet output per plot was significantly greater in the fusil plots (more than four times that of the controls) but only enhanced ($P < 0.07$) in the burn plots. (Further examination of the data showed that one of the five burn plots experienced an anomalous decrease in survivorship and was solely responsible for the lack of a statistical significance of burning on nutlet output per plot. Removing this plot from the analysis allowed the conclusion that burning significantly increased nutlet output per plot when compared with controls ($P<0.05$) For now, however, the data will not be excluded and the more conservative conclusion will be drawn).

Clipping the grasses had the unexpected result of reducing shoot length of *Amsinckia* plants (Table 18) and the degree of branching as well (data not shown). Although not statistically different from the controls, plants in clipped plots were observed to be weaker and never produced showy inflorescences laden with flowers. This was despite the fact that the grass canopy in each clipped plot was low and open throughout the growing season (Tables 9 and 10). As a result, nutlet output per plant was very low and similar to that reported for wild plants at Site 300 (Taylor 1987). Despite the fact that suvivorship was the same as in fusil plots, nutlet output by the clipped plots was just above nutlet input (160, the number sown in each plot) and in one case was much less. It is likely that nutlet predation and less than 100% germination would further restrict the growth and stability of these subpopulations. Clipping is not, therefore, a recommended treatment for reducing competition - in fact, it appears to increase it by some unknown mechanism. Perhaps the grasses respond by producing more robust rootstocks and root systems, usurping underground resources (mineral nutrients, water) more effectively. Regardless, the data strongly call into question the management practice of using livestock for favoring native herbs over introduced grasses in California annual grassland. Even in the absence of trampling and other direct impacts, the simple removal of the grass canopy at this time of year accentuates competition with plants like *Amsinckia*, reducing fecundity and contributing to population decline or instability.

The different treatments produced subpopulations of *Amsinckia grandiflora* within the plots that were easy to distinguish from a long distance away. Fusilade-treated plots were tall and lush with bright orange flowers, each a vibrant patch of color against the green backdrop of annual grasses. Most of the burn plots were also showy, although

the orange color was diluted with tall, leafy grasses. Control and clip plots were not so distinct and more-or-less blended in with the rest of the meager wildflower display in this droughted year. These visual differences were not lost on the pollinators of Lougher Ridge. The open flowers of *Amsinckia* attracted an abundance and variety of native insects, including anthophorid bees, bumblebees, snakeflies, flower beetles, and deerflies. More important, however, was the observation that the showy fusil and burn plots were visited constantly and for long periods of time. Fusil plot 1C, for example, was always found to have considerable activity and individual insects lingered for quite some time before moving on. While in the plot, bees were found to visit between 27 and 45 *Amsinckia* flowers per minute on a number of different individual plants. In contrast, clip plot 1B, just 2 meters away, had long intervals (e.g. 24 minutes) between pollinator visits. Once in the plot, a bee was observed to visit 28 flowers in 40 seconds but then flew away (data collected by Ann Howald, pers. communication, 3/30/90). It is likely, therefore, that the treatments affected the abundance, diversity and activities of pollinators by producing subpopulations with different floral displays.

Comparisons of mean maximum shoot length, mean shoot length and nutlet output per plant between Davis and Site 300 source plants did not yield any statistically significant differences (Tables 15 and 16). Both sources exhibited similar responses to burning (increased plant size), clipping (no effect or slight diminution of plant size and nutlet output) and Fusilade (increased plant size and nutlet output). The small, electrophoretically-detectable differences in allozyme variation did not translate into differences in growth or fecundity during the first year of the new population. This is not to say, however, that the alternative alleles carried by these sources will never have an ecological or evolutionary impact on *Amsinckia grandiflora*.

Table 18. Treatment effects on plant size (length of main shoot) and nutlet production (per plant and per plot) of *Amsinckia* (both sources) during the period following maximum flowering (mid-March to April, 1990). Mean maximum plant size calculated from the 10 largest individuals in each plot. Values (mean ± SD) in a column followed by the same letter are not statistically different from the control (P<0.05, ANOVA unless otherwise indicated).

	plant size		nutlet production	
	mean maximum (cm)	mean (cm)	mean (# / plant)	mean (# / plot)
control	26.0 ± 3.1^{a}	18.6 ± 2.8^{a}	15.1 ± 10.1^{a}	835 ± 606^{a}
burn	33.7 ± 5.3^{b}	22.9 ± 3.2^{a}	29.1 ± 14.4^{a}	2324 ± 1379^{a+}
clip	23.1 ± 3.7^{a}	14.9 ± 2.9^{a}	6.6 ± 5.6^{a}	473 ± 364^{a}
fusil	40.5 ± 4.1^{b*}	28.6 ± 3.3^{b*}	53.5 ± 16.5^{b**}	3522 ± 1274^{b*}

+ P < 0.07 * P < 0.01 ** P < 0.005

Table 19. Treatment effects on plant size (length of main shoot) of *Amsinckia* from Davis and Site 300 sources during the period following maximum flowering (mid-March to April, 1990). Mean maximum plant size calculated from the 10 largest individuals in each plot. Values (mean ± SD) in a column followed by the same letter are not statistically different (P<0.05, ANOVA unless otherwise indicated).

	Davis source		Site 300 source	
	mean maximum (cm)	mean (cm)	mean maximum (cm)	mean (cm)
control	24.9 ± 2.9^{a}	18.4 ± 3.6^{a}	21.1 ± 5.3^{a}	18.0 ± 3.8^{a}
burn	31.2 ± 5.3^{a+}	23.0 ± 3.9^{a}	30.0 ± 5.3^{b*}	22.3 ± 3.6^{a}
clip	22.0 ± 4.2^{a}	14.8 ± 3.8^{a}	18.2 ± 2.8^{a}	13.9 ± 2.7^{a}
fusil	38.7 ± 2.8^{b**}	28.5 ± 3.2^{b**}	35.4 ± 2.8^{b**}	27.8 ± 5.4^{b*}

+ P < 0.07 * P < 0.025 ** P < 0.001

Table 20. Treatment effects on nutlet output (# per plant) of *Amsinckia* from Davis and Site 300 sources during the period following maximum flowering (mid-March to April, 1990). Values (mean ± SD) in a column followed by the same letter are not statistically different ($P<0.05$, ANOVA unless otherwise indicated).

	Davis source	Site 300 source
	mean nutlet output (#/plant)	mean nutlet output (#/plant)
control	15.5 ± 9.8^{a}	13.8 ± 11.3^{a}
burn	30.6 ± 15.2^{a}	27.3 ± 14.1^{a}
clip	7.2 ± 6.7^{a}	4.9 ± 4.1^{a}
fusil	$54.4 \pm 14.4^{b***}$	$52.4 \pm 21.5^{b**}$

* $P < 0.025$ ** $P < 0.01$ *** $P < 0.001$

Evaluation of the Reintroduction Based on Predictions from Existing Data

The predictions in Table 3 were based on studies of *Amsinckia grandiflora* and other herbaceous taxa. As a result, there was great uncertainty and wide ranges of the predicted parameters. Nevertheless, they do provide a simple means of evaluating the reintroduction and the various treatments in relation to assumptions about the species, treaments, and the habitat made at the outset.

As a whole, the reintroduction could be termed a success in its first year because the estimated number of nutlets actually produced (~35, 000) exceeded the maximum predicted (~29,000). Too much weight cannot be assigned to this conclusion because invalid assumptions were built into the predictions (see below) that caused compensating errors. Perhaps the conclusion of success should be based on the fact that the ratio of total nutlet output to input (35,000 / 3,460) shows that the population has the potential of growing by an order of magnitude. The word potential must be emphasized here, because of uncertainties associated with weather, habitat factors, and

site management during the years to come.

With respect to specific management techniques, the predictions were sometimes accurate, sometimes not. Numerically, the actual number of germinules (1774) fell near the midrange of the predicted values. This means that laboratory germination was an adequate predictor of germination *in situ* for this species. However, there was no effect of the treatments on germination, so that the expected range of live germinules should have been the same between control and treated plots. Survivorship to reproduction was greatly underestimated for all treatments (it should have been between 50 and 75%) and so the maximum number of adult plants predicted in Table 3 (896) was short of the actual number which became reproductive (1101 at least). Only the burn treatment had a significant, postive effect on survivorship to reproduction compared to controls and there was no sign of the assumed stress caused by herbicide residuals. Nutlet production was also not accurately predicted. Control plants produced more nutlets at Lougher Ridge than similar, competitively-suppressed plants at Site 300. This may have been due to the greater amount of precipitation received by the former. Fusilade-treated plants produced many more than predicted, indicating that the deliterious effects of competition with annual grasses had been dramatically underestimated. In addition, grass competition was not expected to develop in the burn plots and reduce nutlet output to the degree that it did. The most inaccurate prediction of nutlet output was made for plants in the clip plots. The inhibitory effects of selectively clipping grasses were not anticipated, and the assumption that competition would be weak under these conditions was completely invalid.

This evaluation leads to the conclusion that new populations of *Amsinckia grandiflora* can be created in mesic annual grassland if the habitat is treated to minimize competition with annual grasses. The evaluation also demonstrates that we are not yet able to make very accurate predictions of the demographic characteristics of reintroduced populations or of the effects of certain treatments on the habitat. It stresses the need for additional experimental studies of rare plants and their habitats in order to generate basic data that can be practically applied to specific conservation efforts.

Conclusions and Management Recommendations

1) Regarding the hypotheses in Table 1:

a) The hypothesis that annual grass cover has no effect on the demographic performance of *Amsinckia grandiflora* is accepted with respect to *in situ* germination. It is rejected, however, with respect to mortality rates, survivorship to reproduction, plant size and reproductive output (nutlet production). All of these were significantly influenced by the presence of annual grasses, all to the detriment of the reintroduced population. Therefore, annual grass cover must be controlled in order to promote population growth and stability of this highly endangered plant.

b) The hypothesis that demographic performance of *Amsinckia grandiflora* cannot be affected by manipulating annual grass cover is accepted with respect to the treatment of hand clipping. Although it significantly decreased seedling mortality rates, clipping apparently intensified competition later in the growing season for unknown reasons. *Amsinckia* plants in clipped plots were smaller and produced fewer nutlets than control plants, although the differences were not statistically significant. The results of this experiment indicate that livestock grazing could have a detrimental effect on *Amsinckia* populations even if the effects of trampling and direct consumption were disregarded. Additional experiments on the effects of grazing on this and other native herbs are recommended in order to test assumptions about the use of livestock for managing annual grassland in parks and preserves.

The hypothesis is rejected, however, with respect to the control burn and grass-specific herbicide treatments. Burning significantly reduced mortality rates early in the growing season, significantly increased survivorship to reproduction and maximum plant size, with insignificant enhancement of nutlet output. The effect on reproduction was diminished because of competition with annual grasses that re-established themselves after the burn and grew vigorously late in the season. Spraying with Fusilade had no effect on mortality rates or survivorship to reproduction, but it significantly increased plant size and, therefore, nutlets produced per plant and per plot. The treatment effectively eliminated competition from annual grasses and greatly increased the reproductive output of *Amsinckia grandiflora*. It is recommended,

therefore, that fire and grass-specific herbicides should be viewed as standard tools in the management and recovery of native forbs like *Amsinckia* . Further studies are needed to determine the range of species, habitats, and land-use situations in which these tools can be used safely, responsibly, and effectively to meet conservation objectives.

c) The hypothesis that nutlets from the more genetically variable Site 300 source would not demonstrate better demographic performance than those from the Davis source is accepted. Germination, mortality rates, survivorship to reproduction and nutlet output per plant were the same for all plants regardless of origin. Therefore, the rather small differences in alleles per locus, % polymorphic loci, and heterozygosity per locus for the enzyme systems we characterized had no apparent effect on the fitness of individuals or the genetic structure of the new population (although more data are been generated on the latter). There were, however, some consistent differences between the two sources in response to burning and reproductive phenology that could, after many generations, produce some significant effects at the population level. It is necessary, therefore, to monitor the genetic as well as the demographic characteristics of the new population over long periods of time in order to determine those effects.

2) Regarding the predictions in Table 3:

a) The reintroduction could be termed a success in its first year because the estimated number of nutlets actually produced (~35, 000) exceeded the maximum predicted (~29,000) and the ratio of total nutlet output to input (35,000 / 3,460) shows that the population has the potential of growing by an order of magnitude.

b) The actual number of germinules (1774) fell near the midrange of the predicted values. This means that laboratory germination was an adequate predictor of germination *in situ* for this species. However, there was no effect of the treatments on germination, so that the expected range of live germinules should have been the same between control and treated plots.

c) Survivorship to reproduction was greatly underestimated for all treatments (it should have been between 50 and 75%) and so the maximum number of adult plants predicted (896) was short of the actual number which became reproductive (1101 at least). Only the burn treatment had a significant, postive effect on survivorship to reproduction compared to controls and there was no sign of the assummed stress caused by herbicide residuals.

d) Nutlet production was not accurately predicted. Control plants produced more at Lougher Ridge than similar, competitively-repressed plants at Site 300. This may have been due to the greater amount of precipitation received by the former. Fusilade-treated plants produced many more than predicted, indicating that the deliterious effects of competition with annual grasses had been dramatically underestimated. Related to this was the less-than predicted nutlet output of plants in the burn plants because grass competition was not expected to become important late in the growing season. The worst prediction of nutlet output was made for plants in the clip plots. The inhibitory effects of selectively clipping grasses were simply not anticipated, and the assumption that competition would be weak under these conditions was completely invalid.

3) This evaluation leads to the conclusion that new populations of *Amsinckia grandiflora* can be created in mesic annual grassland if the habitat is treated to minimize competition with annual grasses. The evaluation also demonstrates that we are not yet able to make very accurate predictions of the demographic characteristics of reintroduced populations or of the effects of certain treatments on the habitat. It stresses the need for additional experimental studies of rare plants and their habitats in order to generate basic data that can be practically applied to specific conservation efforts.

4) The question of long-term management of the population has yet to be resolved. Clearly, a combination of controlled burning early in the fall and the use of a dilute, grass-specific herbicide in the late winter would probably provide a substantial boost. The frequency of treatment would depend on the rate of recovery of the annual grasses in habitat patches that were occupied by *Amsinckia*. Perhaps intensive treatment of habitat on a large scale and concurrent restoration of perennial grass cover are required in order to ensure population stability of *Amsinckia grandiflora* within the community.

5) A number of new techniques were successfully employed in this reintroduction effort that could be applied to other endangered taxa. These included;

a) microsite evalution using *in vitro* measures of demographic performance.

b) electrophoretic characterization of the propagule source populations.

c) models for predicting genetic and demographic structure of the reintroduced population (still under testing and development).

d) small-scale burning treatments.

e) precision sowing and monitoring frames for following the fates of thousands of propagules.

Literature Cited

Conkle, M.T., P. D. Hodgskiss, L.B. Nunnally and S.C. Hunter. 1982. Starch gel electrophoresis of conifer seeds: A laboratory manual. U.S.D.A. Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA. General Tech. Report PSW-64. 18 p.

Hamrick, J.L., Y.B. Linhart and J.B. Mitton. 1979. Relationships between life history characteristics and electrophoretically-detectable genetic variation in plants. Annual Review of Ecology and Systematics 10, 173-200.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1, 143-158.

Lesica, P., R.F. Leary, F.W. Allendorf and D.E. Bilderback. 1988. Lack of genic diversity within and among populations of an endangered plant *Howellia aquatilis* . Conservation Biology 2, 275-282.

Nickrent, D.L. 1989. Laboratory procedures for starch gel electrophoresis. unpublished manuscript, available from author, Department of Plant Biology, University of Illinois, Urbana, IL 61801. 24 p.

Ornduff, R. 1976. The reproductive system of Amsinckia grandiflora, a distylous species. Systematic Botany 1, 57-66.

Pavlik, B. M. 1988. Nutlet production and germination of Amsinckia grandiflora. I. Measurements from cultivated populations. California Department of Fish and Game, Endangered Plant Program, Sacramento, CA. 27 pp.

Pavlik, B. M. 1989. 1989 census of *Amsinckia grandiflora* at Site 300. unpublished report submitted to California Department of Fish and Game, Endangered Plant Program, Sacramento, CA.

Pavlik, B.M. and M.G Barbour. 1988. Demographic monitoring of of endemic sand dune plants, Eureka Valley, California. Biological Conservation 46, 217-242.

Pavlik, B. M. and K. Heisler. 1988. Habitat characterization and selection of potential sites for establishment of new populations of *Amsinckia grandiflora* . California Department of Fish and Game, Endangered Plant Program, Sacramento, CA. 17 p.

Pavlik, B. M., N. Ferguson, E. Manning and M. Nelson. 1988. Demographic studies of endemic plants at the Antioch Dunes National Wildlife Refuge. II . Seedling demography, seed production and seed bank dynamics. State of California, Department of Fish and Game, Endangered Plant Program, Sacramento, California. 107 p.

Schwartz, O.A. 1986. Lack of protein polymorphism in the endemic relict *Chrysosplenium iowense* (Saxifragaceae). Proceedings of the Iowa Academy of Sciences.

Taylor, D.W. 1987. Amsinckia grandiflora - 1987 monitoring results. Unpublished memorandum to U.S.F.W.S., Sacramento, CA.

Waller, D.M. 1987. Genetic variation in the extreme endemic *Pedicularis furbishiae* (Scrophulariaceae). Conservation Biology 1, 335-340.

Weller, S. and R. Ornduff. 1977. Cryptic self-incompatibility in Amsinckia grandiflora (Boraginaceae). Evolution 31, 47-51.

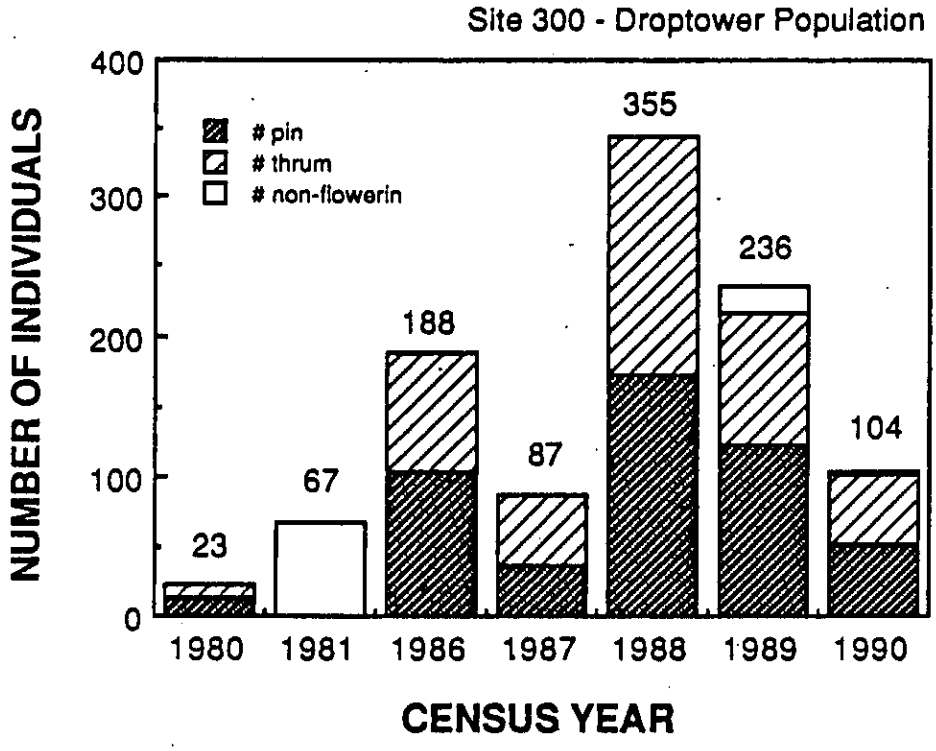

Figure 1. Spring census results for the Droptower Population of Amsinckia grandiflora at Site 300, San Joaquin County, California. Total population size and the proportion of pin and thrum individuals are shown.

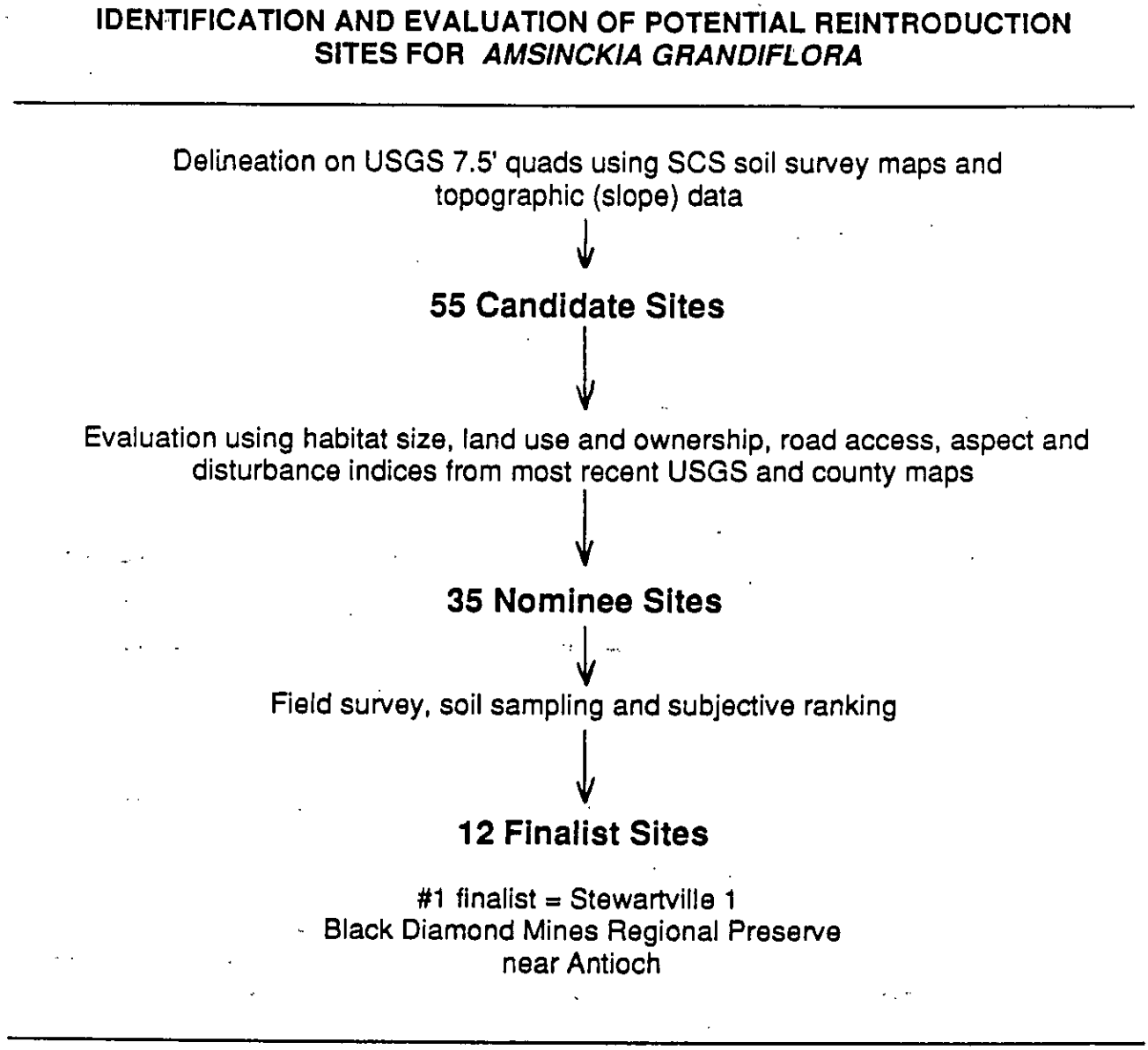

Figure 2. Identification and evaluation of potential reintroduction sites for Amsinckia grandiflora. The Lougher Ridge microsite was eventually selected from a field of six at the Stewartville 1 finalist site. From Pavlik and Heisler 1988.

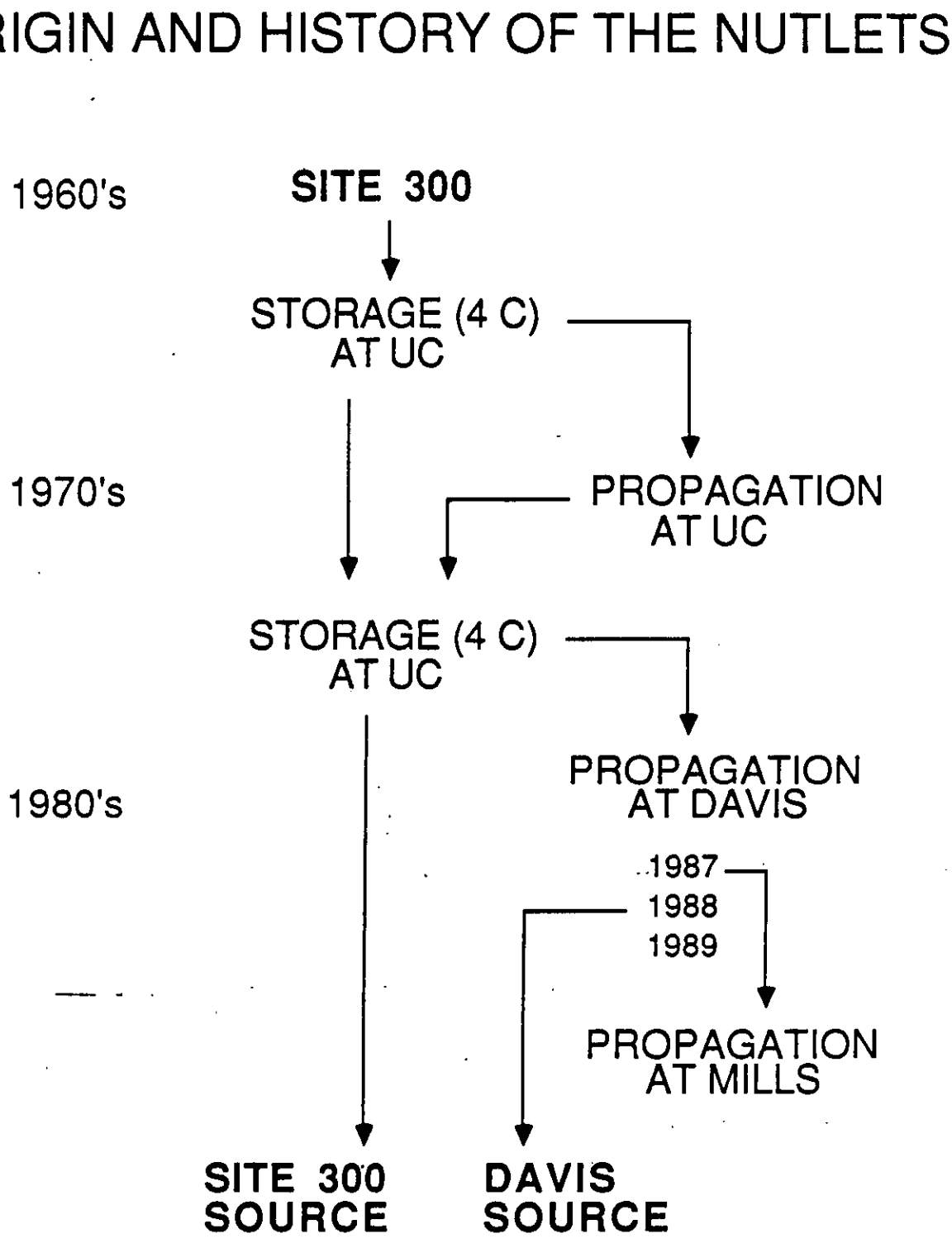

Figure 3. Origin and history of the nutlets used in the reintroduction.

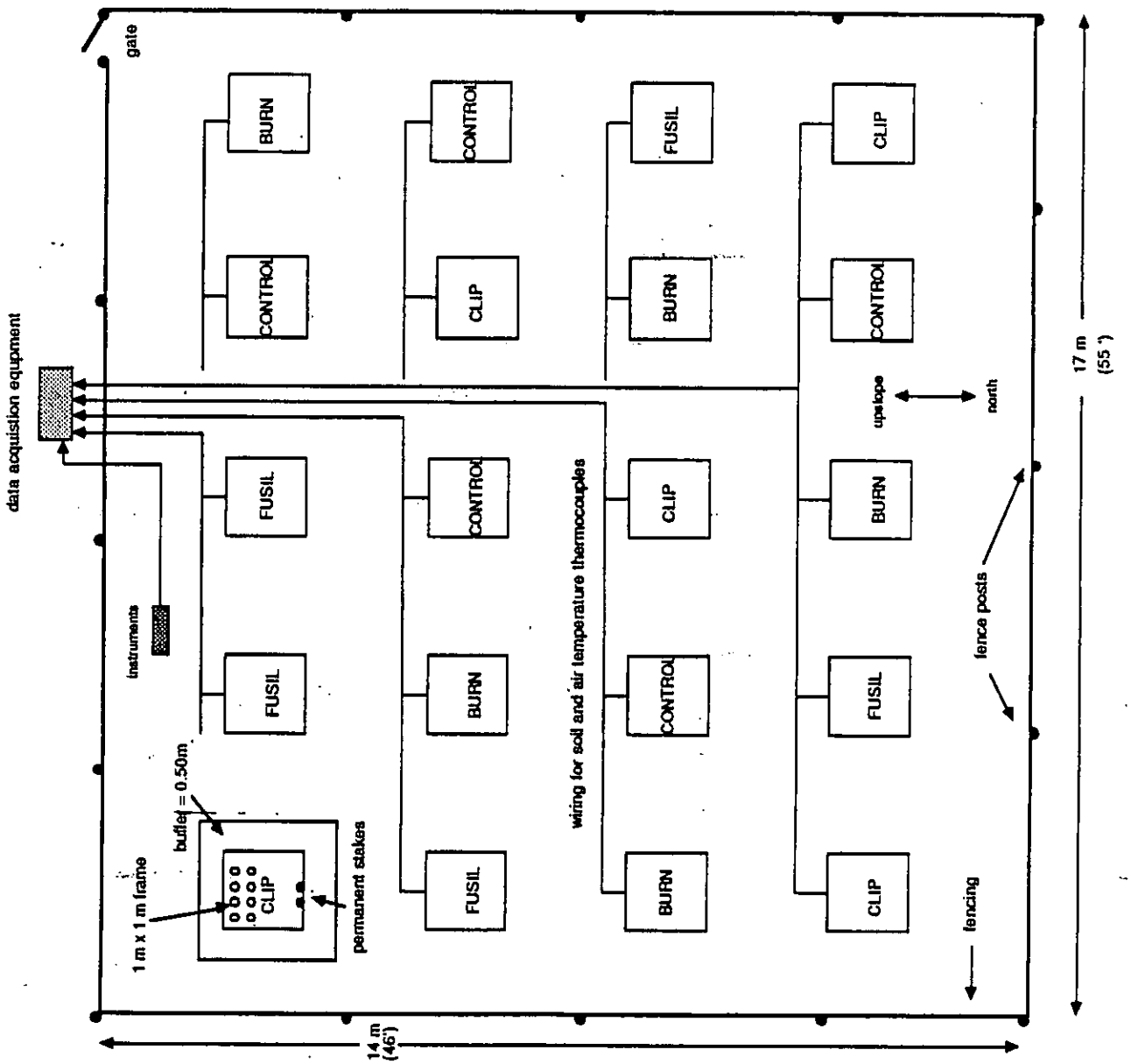

Figure 4. Layout of plots at the Lougher Ridge microsite. Treatments were randomly located.

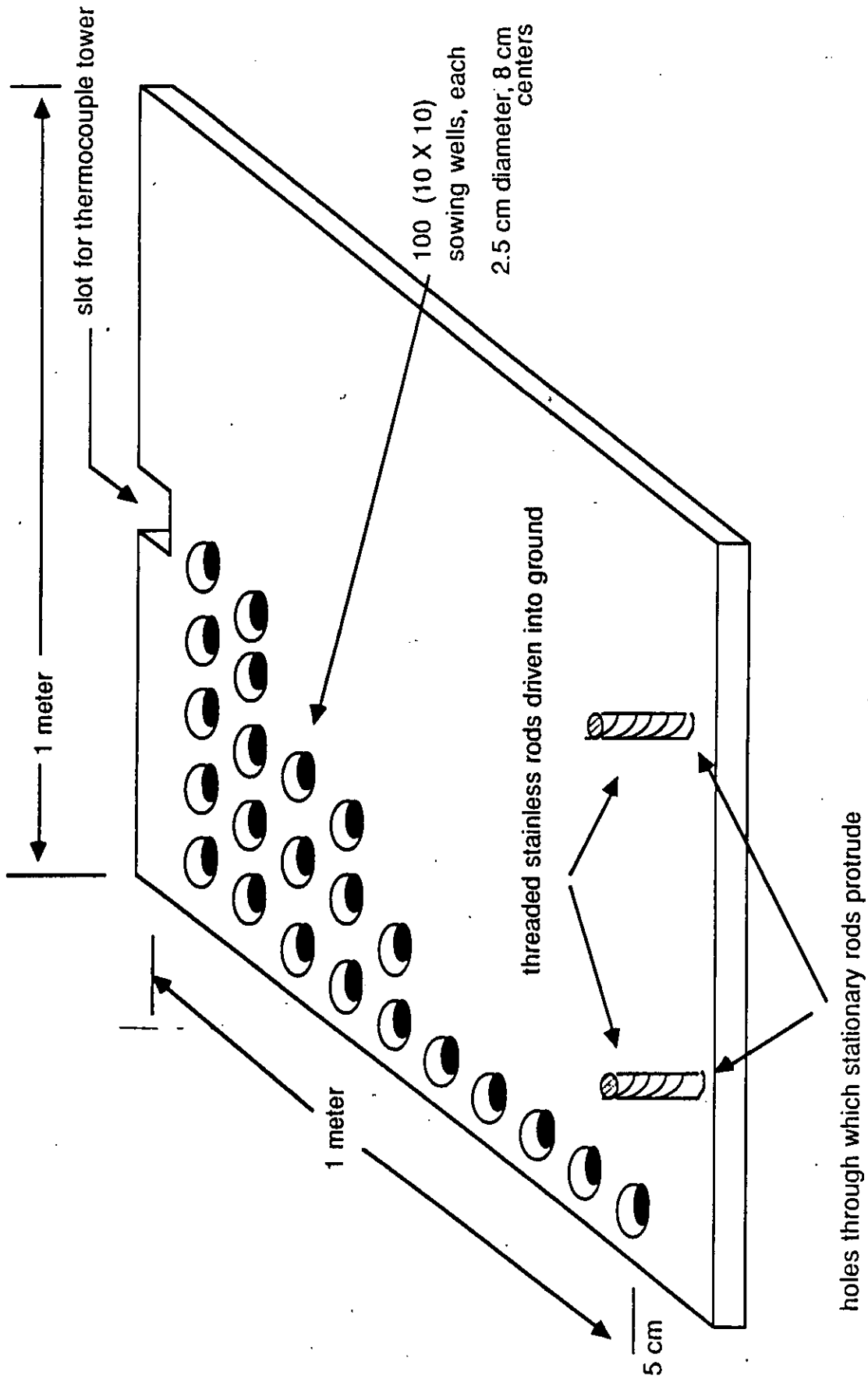

Figure 5. 100 hole frame for monitoring the seeds and seedlings of Amsinckia grandiflora.

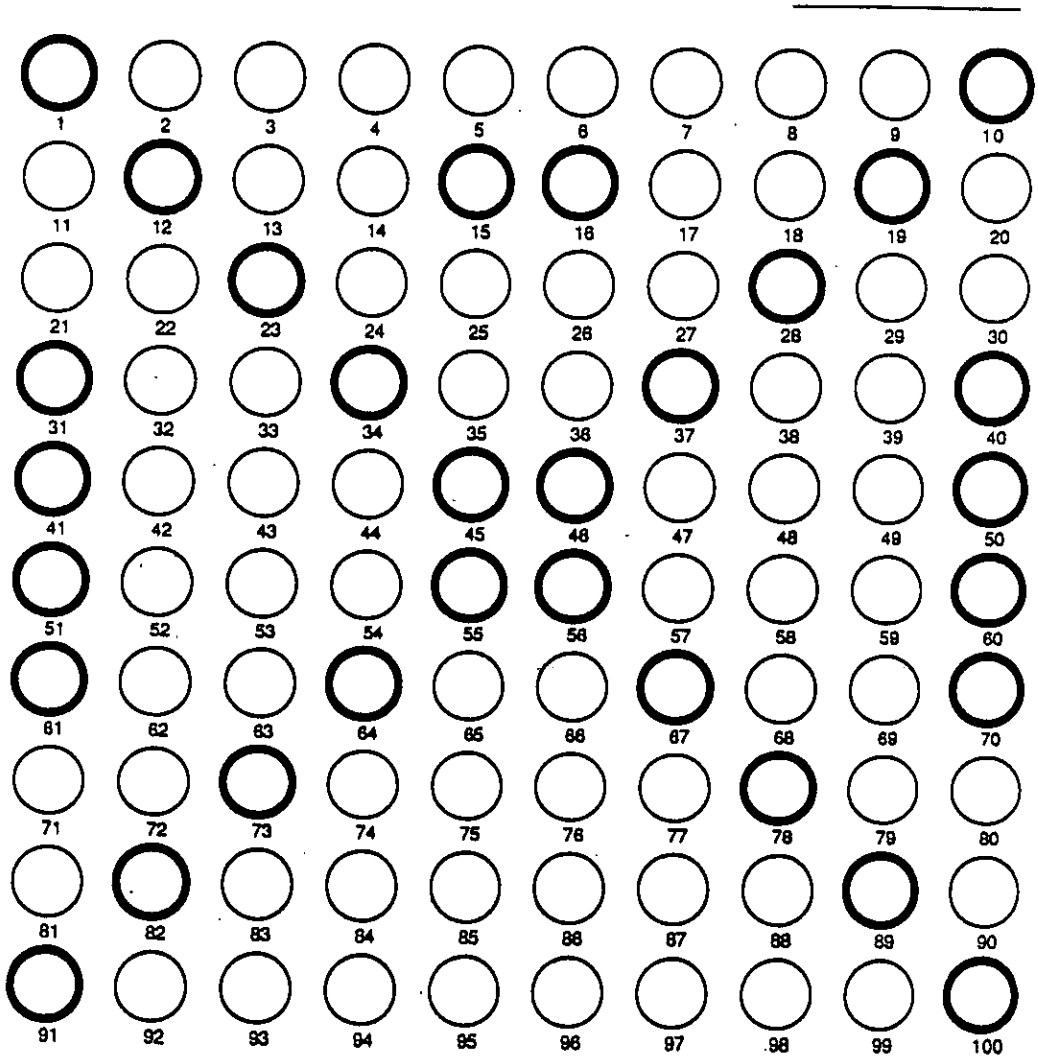

Figure 6. Sample data sheet used for demographic monitoring. Each plot had its own data sheet on every sampling date.

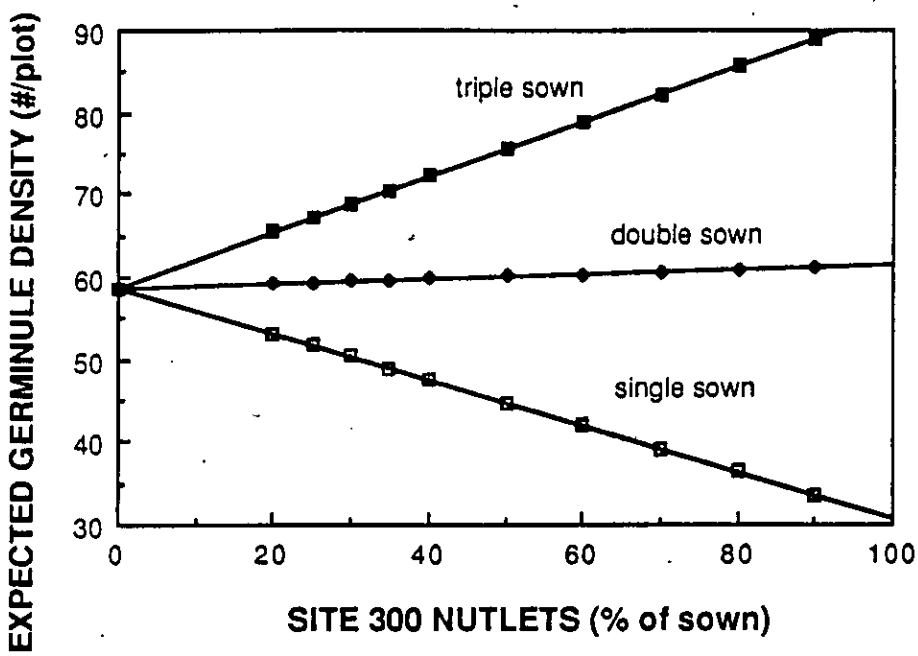

Figure 7. Germinule density as a function of source composition of founding nutlets and sowing density per well.

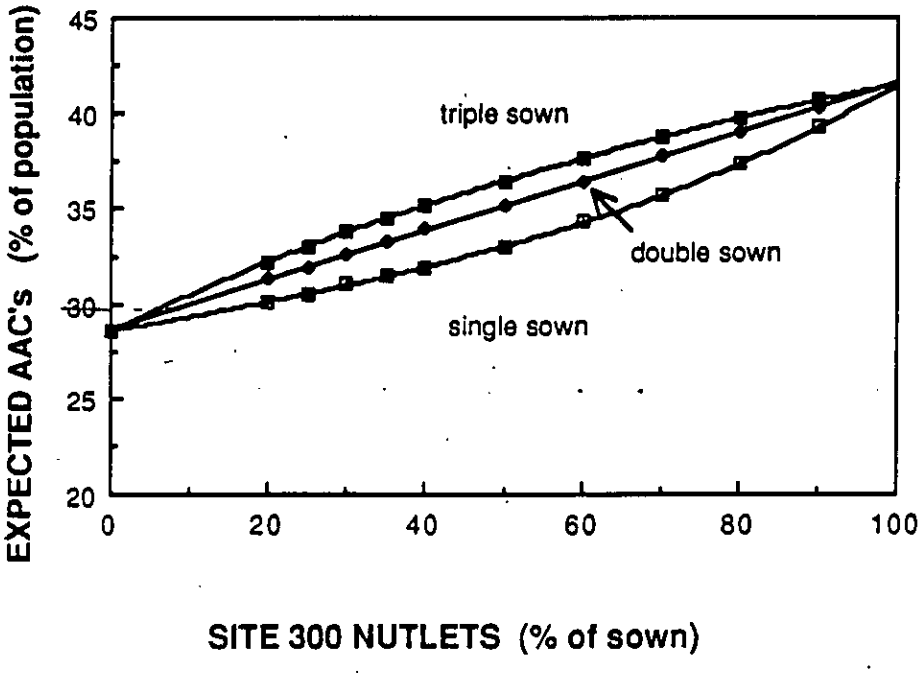

Figure 8. Number of alternative allele carriers (ACC's) as a function of source composition of founding nutlets and sowing density per well.

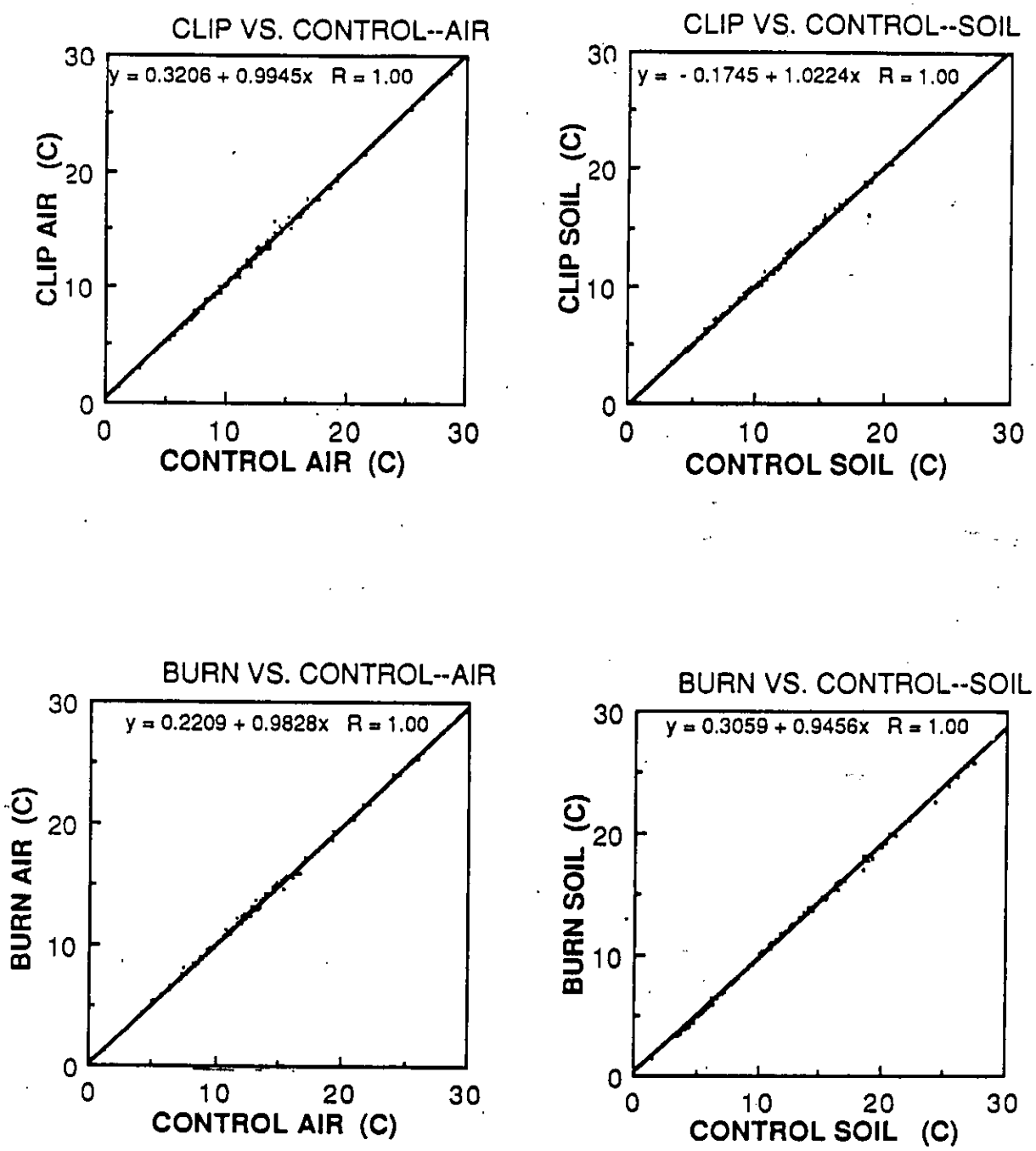

Figure 9. Comparisons of air and soil temperatures in clipped and burned plots. Each point represents mean daily temperature (n=48 measurements per day) as recorded by data acquisition equipment and thermocouples in every plot. Slopes and R values close to 1.0 indicate that the treatments did not significantly alter plot microclimate.

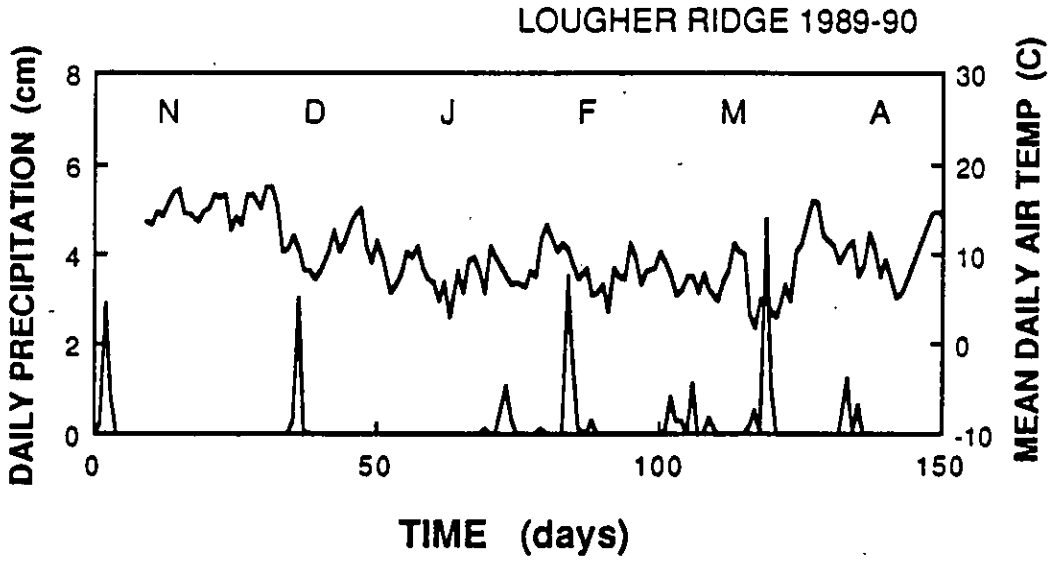

Figure 10. Mean daily air temperature (n=48 measurements per day) and total daily precipitation at the Lougher Ridge microsite, October 1989 to April 1990. At total of 33.40 cm (=11.39 inches) was received between October 20, 1989 (day 1) and May 7, 1990 (day 198). Amsinckia grandiflora was active when 28.07 cm (=11.05 inches) was received (day 0 just prior to germination and until day 180).

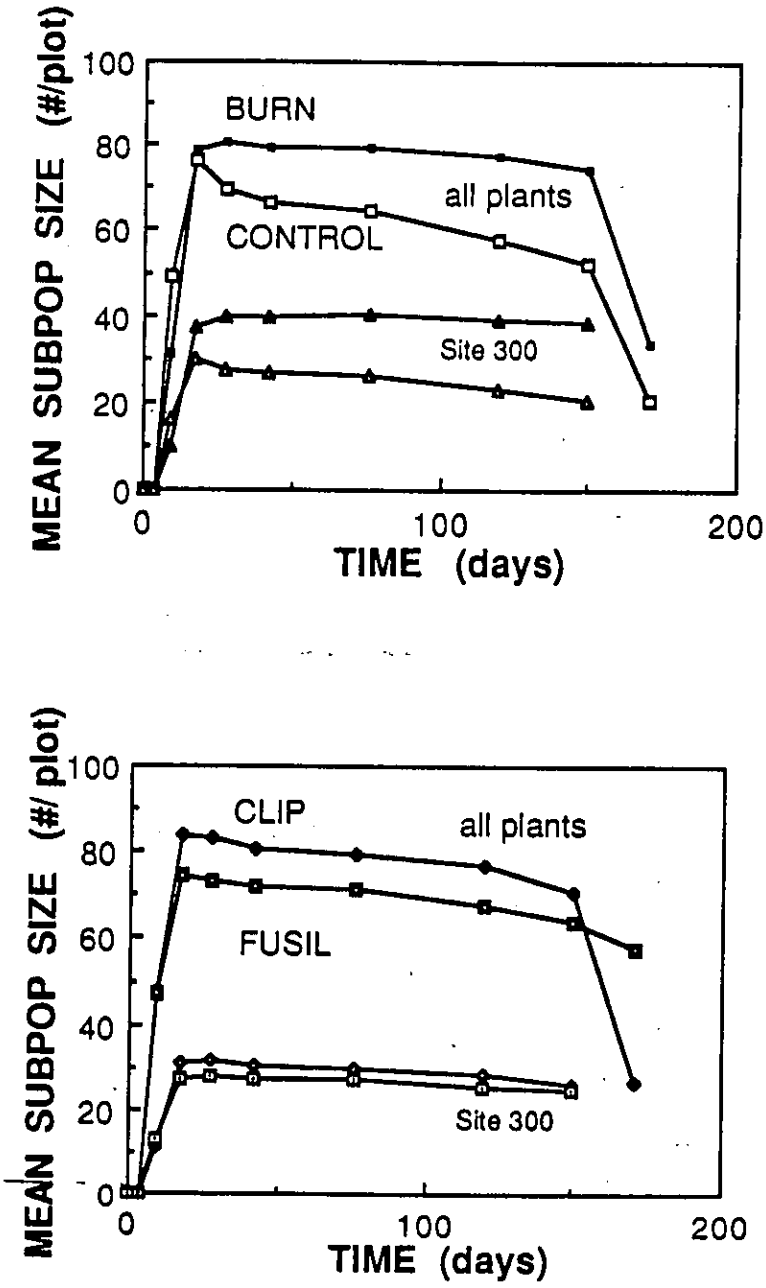

Figure 11. Mean sizes of the subpopulations within treatment and control plots, October to April. Total plants (both nutlet sources) and plants from the Site 300 source are shown.

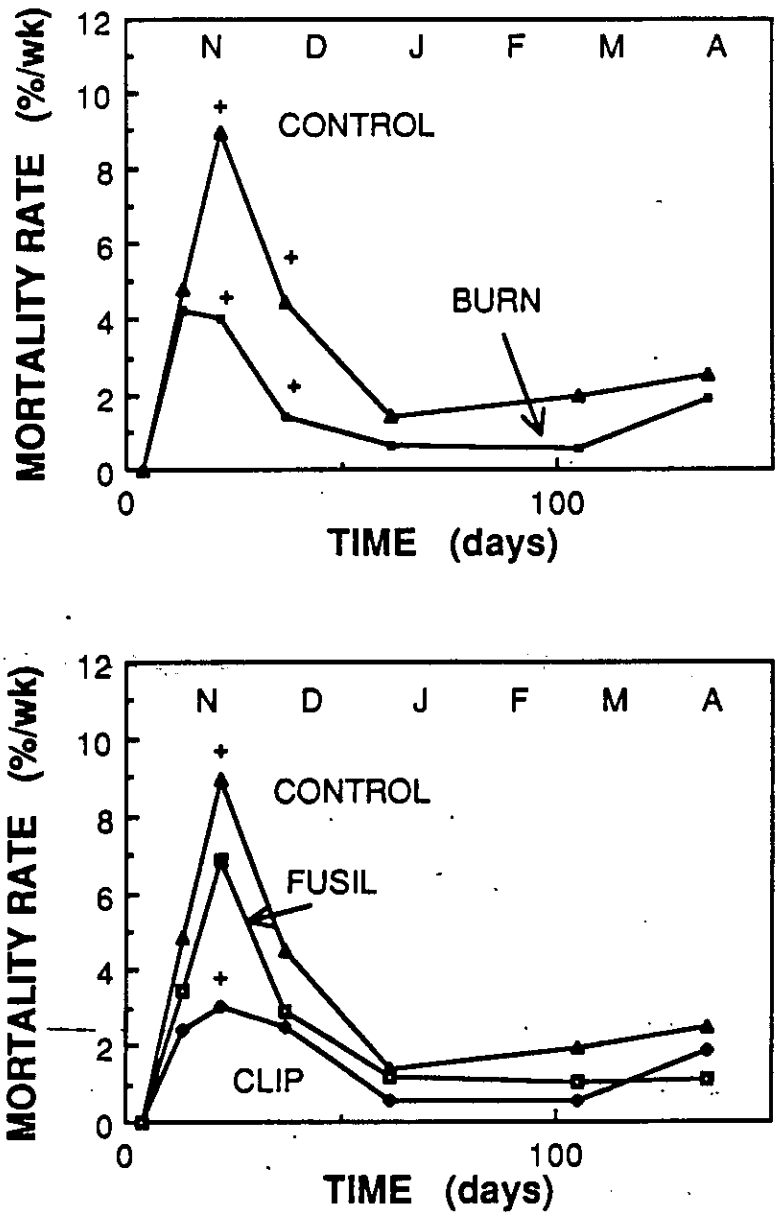

Figure 12. Mortality rate of Amsinckia grandiflora within treatment and control plots, October to April. "+" indicates significantly different from control on a particular date (ANOVA, P 0.05).

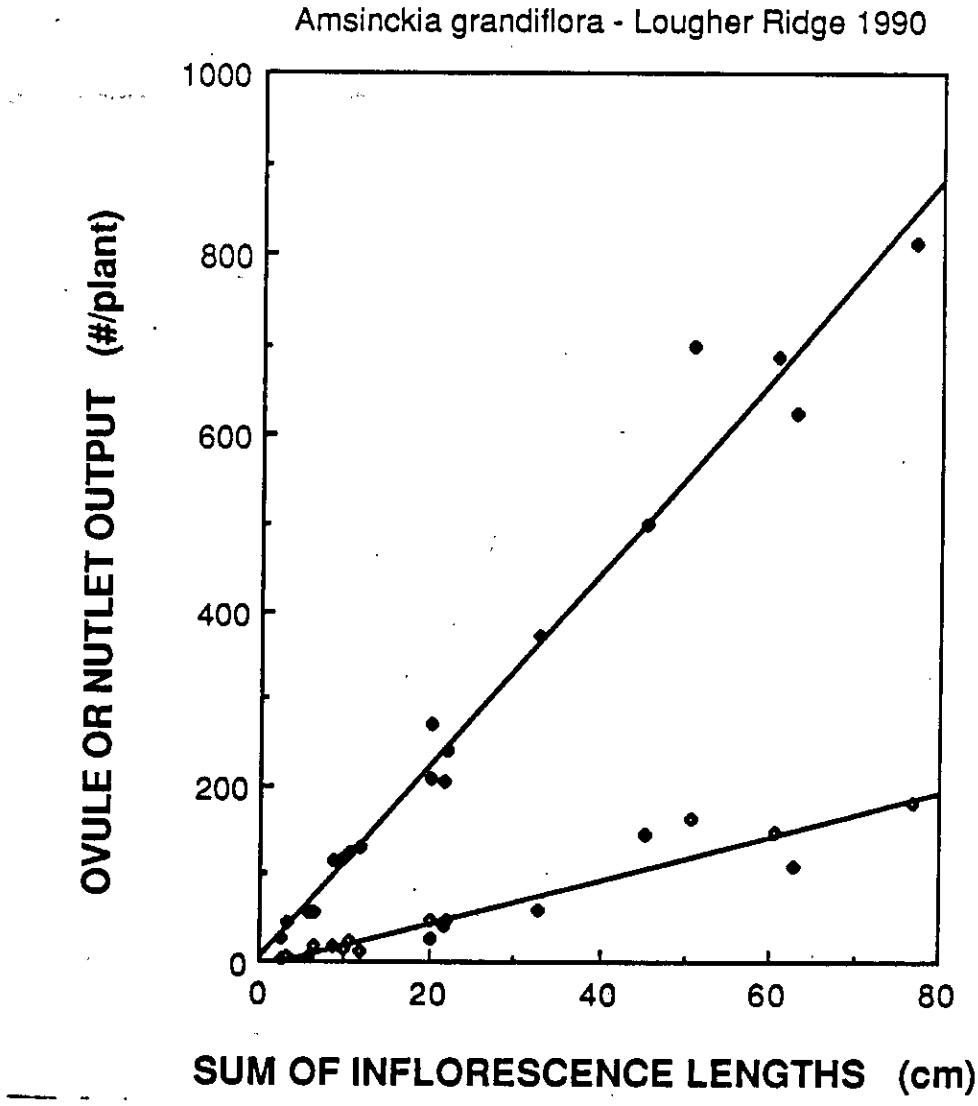

Figure 13. Ovule (upper line, closed symbols) and nutlet (lower line, open symbols) output for Amsinckia grandiflora at Lougher Ridge, March 1990. n=18 plants.

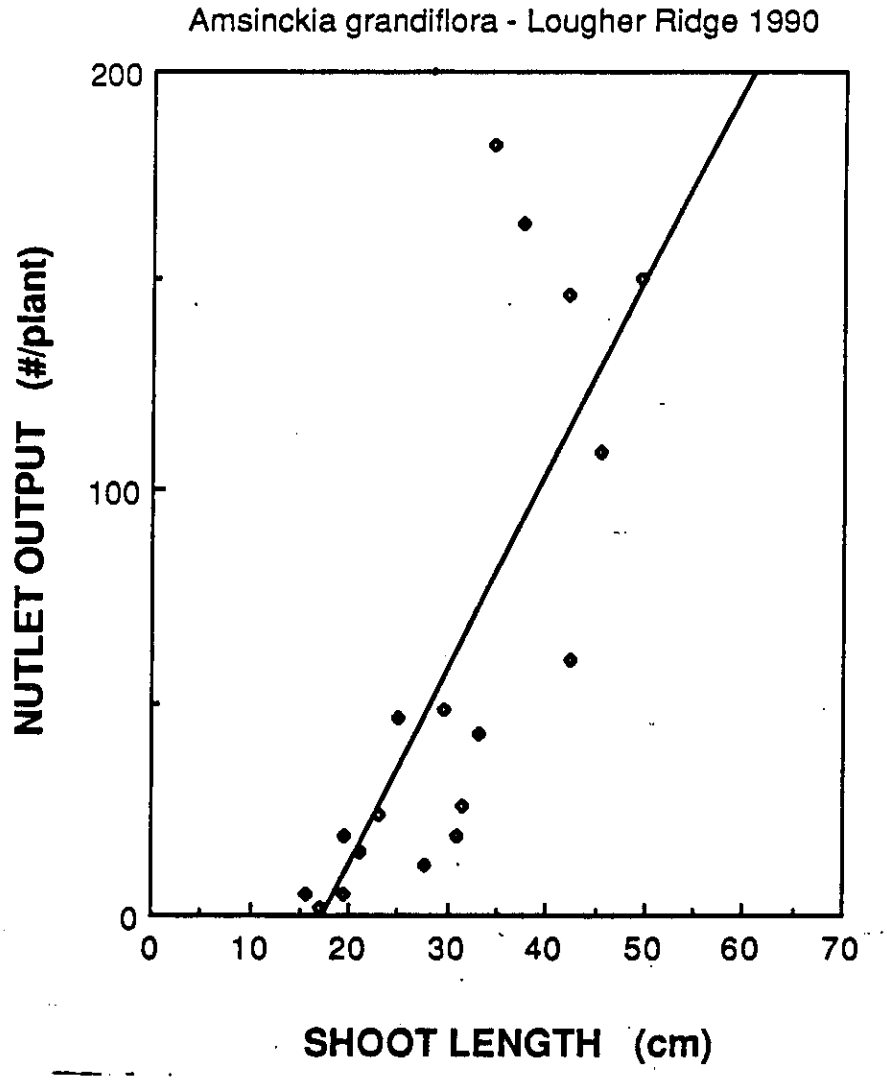

Figure 14. Nutlet output as a function of shoot length for Amsinckia grandiflora at Lougher Ridge, March 1990. See Table 17 for line equation. This relation was used to estimate nutlet output of the population.